



Phytochemical Screening and Antiulcer Activity, of *Ficus thonningii* (Moraceae) Aqueous Fruits Extract in Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors UPU, FCN, TEF and JDF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NNB, NVN, TOY, NEM, NB, MG, IM and ABN managed the analyses of the study, data mining and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The World Health Organization (WHO) has estimated that about 80% of the world's population rely mostly on traditional medicine. Peptic ulcer disease (PUD) which results from an imbalance between stomach acid-pepsin and mucosal defense barriers is a chronic disease affecting up to 10% of the world's population and represents 33% of gastroenterology consultations in Cameroon. The people in low medium income countries depend mostly on medicinal plants for primary healthcare since they can be accessed quickly and are affordable. Such plant is *Ficus thonningii* Blume, which is found abundantly in Africa and also in Cameroon. The objective of this study was to phytochemically screen the aqueous fruits extract of *Ficus thonningii* (AEFFt), and investigate their antiulcer activity.

Methods: The aqueous fruits extract was phytochemically screened following standard qualitative methods. Four *in vitro* tests to characterize antacid properties were carried out. Ulcers were induced using an ethanol and hydrochloric acid (HCl/EtOH) solution. Ulcer preventive (anti-ulcer) activity was investigated in 6 different treatment groups: 2 conventional drugs (Maalox 100 mg/kg, Omeprazole 20 mg/kg), three doses of AEFFt at (125 mg/kg, 250 mg/kg, & 500 mg/Kg), and a vehicle treatment group (administered only the ulcerogenic agent). Histological analysis of the stomachs was carried out.

Results: The phytochemical screening of the aqueous extract of fruits of *F. thonningii* showed the presence of flavonoids, mucilages, saponins, gallic tannins, betacyanins, and total polyphenols. Following the *in vitro* tests, we obtained a value of 7.4mEq for acid neutralization capacity (ANC) for the extract, and a pH of 4.2 for the extract following FDA test. For the *in vivo* tests, the aqueous extract of fruits of *F. thonningii* (AEFFt) showed a dose-dependent increase ulcer-preventive (gastroprotective) activity with the three treatment aqueous extracts doses.

Conclusion: The study showed that, the aqueous extract of fruits of *Ficus thonningii* showed a dose-dependent ulcer-preventive activity that could be accounted for by the presence of bioactive phytochemicals like polyphenols (flavonoids, tannins).

Keywords: Peptic ulcer disease (PUD); aqueous fruits extract; *Ficus thonningii*; ulcer-preventive activity.

1. INTRODUCTION

The World Health Organization (WHO) defines traditional medicine as the sum total of the knowledge, skills, and practices based on the theories, beliefs, and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement, or treatment of physical and mental illness [1,2]. Medicinal herbs are significant sources of synthetic and herbal drugs [2,3]. The usage of medicinal plants in healing numerous diseases is as old as human beings, and well-known as phytotherapy [2,4]. About 75-90% of the world population still relies on medicinal plants as part of the primary health care, especially in rural communities of Africa and other low medium income countries [5].

Nowadays, herbal medicine or traditional therapeutics which is medical practice that includes diagnosis, prevention and treatment, relying on practical experience and observations handed down from generation to generation, whether verbally or in writing, is becoming a

viable alternative treatment over the commercially available synthetic drugs due its lower cost, perceived effectiveness, availability as well as little or no adverse effects [5,6].

The importance of the study of substances obtained from plants cannot be overstated. Many conventional drugs used in therapy are obtained or conceptualized from plants sources [7]. The WHO's goal of "Health for all" would not be met without the contribution of herbal medicine [7]. These studies are even more important in third world countries where the economic condition necessitates that a lot of the people depend on locally collected and prepared medications due to their quick accessibility, affordability, limited access to orthodox healthcare or low financial capability. In the African sub-region, there is availability of a vast number of naturally occurring medicinal plants [7] such as *Ficus thonningii*.

Ficus thonningii Blume (the common wild fig) is a traditionally important plant species with both nutritional and therapeutic benefits [8]. The

leaves are used for the treatment of diarrhea, gonorrhoea, diabetes, jaundice, scabies, wounds, bronchitis, urinary tract infections, urinary schistosomiasis, gastric ulcers and colitis. The stem bark is used for treating colds, arthritis, inflammation, pneumonia, bronchitis, diarrhea, constipation, bowel disorders and to stimulate lactation [8]. Work on the figs (fruits) and roots of *Ficus thonningii* has led to the discovery of two new flavonoids which with other known compounds were shown to have antioxidant and antimicrobial activity in 2015 by Fongang et al. [9]. The presence of phytochemicals like flavonoids as well as others display several pharmacological properties as gastroprotective bioactive agents [4]

Peptic ulcer describes a group of ulcerative disorders that occur in areas of the upper gastrointestinal tract (GIT) that are exposed to acid-pepsin secretions. The two most common forms of peptic ulcer are duodenal and gastric ulcers [10,11]. A peptic ulcer can affect one or all layers of the stomach or duodenum.

The term 'peptic ulcer' describes a condition in which there is a discontinuity or breach in the entire thickness of the gastric or duodenal mucosa that persists as a result of acid and pepsin in the gastric juice [12]. Peptic ulcer disease (PUD) differs from gastritis, and erosions in that ulcers are larger (greater than or equal to 5 mm) and extend deeper into the muscularis mucosa [13]. The three common forms of peptic ulcers can be grouped according to their etiology: *Helicobacter pylori*-positive, nonsteroidal anti-inflammatory drug (NSAID)-induced, and stress-related mucosal damage (SRMD) [14].

About 10% of the population in developed countries is likely to be affected at some time by peptic ulcer, with the prevalence for active ulcer disease being about 1% at any particular point in time with duodenal ulcers being five times more common than gastric ulcers. Mortality rates are higher among those older than or 65 years and in males compared to females [12,15]. The prevalence of PU is a reflection of *H. pylori* infection prevalence, increasing with NSAIDs and ASA use, as also with ageing population [3].

There exist as of date many conventional drugs used to treat PUD with treatment aimed at relieving ulcer pain, healing the ulcer, preventing ulcer recurrence, and reducing ulcer-related complications [14]. However, coupled with widespread use of nonsteroidal anti-inflammatory

drugs (NSAIDs) and low-dose acetylsalicylic acid, most of these regimens are not fully effective, produce numerous adverse effects, unavailable, inaccessible and costly, resulting in impaired quality of life, work loss, and high-cost medical care [4,14].

Given that the above burden of PUD remains a relevant issue, many populations in rural communities in Africa and other developing regions resort to traditional medicine of which plant drugs form the basis and their use is extensive, increasing and complex [5,6]. Investigations of the new pharmacologically active agents through the screening of different plant extracts led to the discovery of effective and safe drugs with gastroprotective activity. Especially, plants with antioxidant capability as the main mechanism are used as the herbal reservoir for the treatment of ulcer disease [16]. In the constant search for new remedies for PUD, fruits of *Ficus thonningii* Blume were chosen to explore their antacid, antiulcer characteristics.

The treatment and prevention of acid-related disorders are accomplished by decreasing gastric acidity and enhancing mucosal defense. The proximal part of the duodenum is protected from gastric acid through the production of bicarbonate, primarily from mucosal Brunner glands [17,18]. Antacids are prescribed for symptomatic relief of hyperacidity associated with peptic ulcer, gastritis, gastric hyperacidity, which on ingestion react with HCl of gastric juice via neutralization reaction to lower the acidity of the gastric contents [9,19]. Antacids are substances that react with acid in the stomach and ideally, raise the pH of the stomach contents to between 4-5 [14,19].

2. MATERIALS AND METHODS

2.1 Study Design

An experimental *in vitro* and *in vivo* study carried out on *Wistar strain* albino rats.

2.2 Period and Study Site

This study was carried out in the Laboratory for Preclinical Animal Studies and Pharmacotoxicology Research of the Faculty of Medicine and Biomedical Sciences, FMBS, University of Yaoundé I from December 2018 to May 2019.

2.3 Ethical Considerations

Ethical approval was obtained from the institutional review board of the Faculty of Medicine and Biomedical Sciences. Authorization was obtained from the administration of the FMBS, to work in the said laboratory of the faculty. The OECD Guidelines 423 for the use of animals in preclinical studies were observed.

2.4 Plant Material – *Ficus thonningii* Fruits

2.4.1 Harvesting and identification

These fruits were harvested in Bafoussam the West region of Cameroon in January 2019 and taxonomically identified and authenticated at the National Herbarium of Cameroon as whole fruits of *Ficus thonningii* by Mr Tadjouteu F. with the following identification code 44042/HNC. The fruits were washed and dried in a shade, between sheets of newspapers. When dried, the fruits were ground, to get fine powder and stored in airtight containers.

2.5 Preparation of Fruits Extract

2.5.1 Extraction method: maceration

The aqueous extract was prepared by pouring 2000 mL of cold distilled water on 200 g of dried powder of *Ficus thonningii* whole fruits in the ratio 1: 10 (w/v), that is 200 g of powder in 2000 mL of water, giving a 10% w/v drug solution. The mixture was allowed to stand for 48 hours under frequent agitation with the aid of an electronic magnetic stirrer. The mixture was then strained and the marc (the damped plant material) was pressed and the combined liquids were filtered using Whatman paper number 2. When this was done, we then evaporated the filtrate inside an autoclave and measured the yield, with respect to the powder initially used [17].

Extraction yield in % =

$$\frac{\text{weight of extract of } F.\textit{thonningii} \text{ fruits}}{\text{weight of powdered fruits}} \times 100$$

2.6 Phytochemistry of Aqueous Extract of *Ficus thonningii* Fruits (AEFFt)

The fig plant possesses a plethora of phytoconstituents which have been shown by very many research works by different authors.

For the purpose of our study, we carried out the following phytochemical tests.

2.6.1 Test for tannins

About 0.5 g of each portion of the aqueous extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution was added to 2 ml of the filtrate; occurrence of a blue-black, green or blue-green coloration indicated the presence of tannins [20-22].

2.6.2 Differentiation of catechic and gallic tannins

It was obtained by STIASNY reaction, in which: to 30 mL of infused solution, we added 15 mL of STIASNY reagent (10 mL of 40% formalin + 5 mL of concentrated HCl) and heated for 15 minutes in a water bath at 90°C. The appearance of a precipitate indicated the presence of Catechic tannins. After filtration, filtrate was saturated with powdered sodium acetate, then 1 mL of a solution of 1% ferric perchloride (FeCl₃) was added. The presence of gallic tannins not previously precipitated by the STIASNY reagent was indicated by the development of a dark blue shade.

2.6.3 Identification test for phlobotannins

To 1 mL of the plant extract in a test tube, we added a few drops of hydrochloric acid. This mixture was put in a water bath containing water at 100°C and heated for 10 minutes. The appearance of a red precipitate indicated the presence of phlobotannins.

2.6.4 Liebermann-Burchard test for steroids

To 0.2 g of each portion, 2 ml of acetic anhydride was added, the solution was cooled in ice followed by the careful addition of conc. H₂SO₄. Color appearance from violet to blue or bluish-green indicated the presence of a steroidal ring i.e. aglycone portion of cardiac glycoside [20,22-25].

2.6.5 Test for terpenoids

A little of each portion is dissolved in ethanol. To the aqueous extract, 1ml of acetic anhydride was added followed by the addition of concentrated H₂SO₄. Formation of blue, green rings showed the presence of terpenoids [20,21,25].

2.6.6 Test for saponins

5 mL of distilled water was mixed with the plant extract and put in a test tube and violently shaken for about 30 seconds. The test tube was then allowed to stand. If the foam persisted for up to 15 minutes, it indicated the presence of saponins. If the foam was ≥ 1 cm, it indicated an abundance of saponins [21,22].

2.6.7 Identification test for betacyanins

2 mL of the extract was put in a test tube. 2 mL of 2N NaOH were added and the tube heated in a hot water bath at 100°C for 5 minutes. The appearance of a yellow coloration indicated the presence of betacyan.

2.6.8 Tests for flavonoids

2.6.8.1 Shinoda's test for flavonoids

About 0.5 g of each portion of plant extract was dissolved in ethanol, warmed and then filtered. Three pieces of magnesium chips were added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple coloration indicates the presence of flavonoids [23-24].

2.6.8.2 Ferric chloride test for flavonoids

About 0.5 g of plant extract was boiled with distilled water and then filtered. To 2 ml of the filtrate, few drops of 10% ferric chloride solution were added. A green-blue or violet coloration indicates the presence of a phenolic hydroxyl group [20,23].

2.6.8.3 Lead ethanoate test for flavonoids

A small mass of each portion was dissolved in water and filtered. To 5ml of each of the filtrate, 3 ml of lead ethanoate solution was then added. Appearance of a buff-colored precipitate indicated the presence of flavonoids [20,23].

2.6.8.4 Sodium hydroxide test for flavonoids

A small quantity of the extract was dissolved in water and filtered; to this 2 ml of the 10% aqueous sodium hydroxide were added to produce a yellow coloration. A change in color from yellow to colorless upon addition of dilute hydrochloric acid was an indication for the presence of flavonoids [20,22].

2.6.9 Test for alkaloids

A small quantity of the plant extract was stirred with 5 ml of 1% aqueous HCl on water bath and

then filtered. From the filtrate, 1 ml into 3 test tubes. To the first portion (2 mL of extract), few drops of Wagner's reagent (1.27 g of I_2 + 2 g of KI for a final volume of 100 mL) were added; occurrence of a creamy white or reddish-brown precipitate was taken as positive for alkaloids [22,24]. To the second 1ml of extract, 3-5 drops of Mayer's reagent(1.36 g of $HgCl_2$ + 5 g of KI for a final volume of 100 ml) was added and appearance of buff-colored (creamy white) or white-yellow precipitate was an indication for the presence of alkaloids [24]. To the third 1ml of extract, 3-5 drops of Hager's reagent (saturated solution of picric acid) were added and appearance of ayellow precipitate indicated the presence of alkaloids [24].

2.6.10 Test for polyphenols

2.6.10.1 Ferric chloride test

To 2 ml of extract solution, 2-3 drops of $FeCl_3$ were added and appearance of a greenish-blue or black colouration confirmed the presence of polyphenols [22].

2.6.10.2 Lead acetate test

To 2 ml of extract, few drops of lead acetate were added and an appearance of a white precipitate indicated the presence of polyphenols [20].

2.6.11 Identification test for resins

In a test tube containing 1 mL of the extract, we added a few drops of solution of anhydrous acetic acid and 1 mL of sulfuric acid (H_2SO_4). The appearance of a purple colour which rapidly changes to violet colour indicated the presence of resins [20,24].

2.6.12 Oxalates identification test

In a test tube, containing 1 mL of the 1% extract a few drops of ethanoic acid were added. Obtaining a greenish-black color indicated the presence of oxalates.

2.6.13 Identification test for Mucilages

To 1 ml of extract solution, 5 ml of absolute ethanol were added and appearance of a flaky precipitate indicated the presence of mucilages.

2.6.14 Identification test for cardiac glycosides (Keller-Kiliani test)

1 ml of extract were mixed with 3 ml of glacial acetic acid and few drops of ferric chloride

followed by addition of 2 ml conc. H₂SO₄. The formation of a greenish or brown ring at the interface indicated a positive test [22,24].

2.6.15 Identification test for Quinones

To 1 ml of extract, 2 ml of conc. H₂SO₄ were added. The observation of a red colouration indicated a positive test.

2.6.16 Identification test for anthocyanins

2 ml of aqueous extract were added to 2 ml of 2N HCl and ammonia. The appearance of pink-red which turned to blue-violet indicated the presence of anthocyanins [25].

2.6.17 Test animals

The experiments were carried out on adult *Wistar* strain (*Rattus norvegicus*) albino rats from the animal house of FMBS.

2.6.17.1 Identification

By cage card and corresponding bold marker body markings and they were maintained in the animal house of CHUY.

2.6.17.2 Selection of animals

Wistar strain (*Rattus norvegicus*) albino rats were used. All animals used were bred in the FMBS animal house under favorable conditions of 12h of light and 12 h of day. The rats were aged between 7 and 12 weeks, with average weight 177±37.67 g for the antiulcer activity. Both male and female rats were used for the toxicity studies, with average mass 124 ±28.5 g and 87± 27 respectively.

The animals were fed with a diet, consisting of corn meal (45%), wheat flour (20%), fish meal (20%), soybean meal (10%), palm kernel (5%), bone flour for calcium intake (0.98%), cooking salt (0.5%) and vitamin complex (0.5%). They were also allowed free access to regular tap water.

2.6.18 Accommodation of rats

For each study, the animals were separated in different cages, with distinct and clear labels. The cages were made of plastic material with stainless steel grill tops and a space for food and water was made available. The floors were lined with saw dust/or paddy husk to keep it dry. In conditions where the rats had to be starved, they were put in metabolic cages made of stainless steel material with spaced bars, allowing the

feces to fall through, thus preventing them from eating their feces. In each cage, the tails of rats were marked with bold markers, with the number of lines denoting the rat number. These animals were then crosschecked to make sure that they were in good health and kept in natural environmental conditions (12 h of light and 12 h of darkness). Each day, the rats were fed with the above mentioned meal and given water.

2.6.19 Dosing of plant test extracts

The ulcerogenic agent, reference drugs or aqueous fruits extract were administered by oral gavage using an intubation needle, fitted into syringes of different volumes. These substances were calculated and given to the rats according to their individual weights [25].

The *Wistar* rats were divided into six groups of 5 animals each. The first group was one that received ulcerogenic substances without pretreatment with plant extract. The second, third, and fourth groups of rats received a pretreatment of aqueous extract of plant at various doses then the ulcerogenic substance or solution. The fifth and sixth groups received the ulcerogenic substances after pretreatment with an existing antiulcer, as a comparative model. The reference antiulcer drugs used in this experiment were Omeprazole 20 mg of batch number PBEH0042 and Maalox 400 mg with batch number U650 both bought from a Yaoundé-based community pharmacy.

2.7 In vitro Antacid Activity

2.7.1 Acid neutralizing capacity (ANC)

This method was adopted from the USP 29 guide for measurement of the ANC. The one mentioned here was unadulterated. The acid neutralizing capacity (ANC) of an antacid is the amount of acid that it can neutralize. This ANC was measured in the laboratory by a process known as back titration. This involved dissolving the antacid in an excess of acid and then titrating the acidic solution against a known concentration of base until the endpoint is reached.

The moles of acid neutralized equals the difference between the moles of acid added and the moles of base required for the back titration. For this investigation:

$$\begin{aligned} &\text{Moles of acid neutralized} \\ &= \text{moles of HCl added} - \text{moles of NaOH required} \\ &= (\text{Volume}_{\text{HCl}} \times \text{Molarity}_{\text{HCl}}) - (\text{Volume}_{\text{NaOH}} \times \text{Molarity}_{\text{NaOH}}) \end{aligned}$$

$$\text{Acid neutralizing capacity per gram of antacid} = \frac{\text{moles of HCl neutralized}}{\text{grams of antacid}}$$

The samples of the aqueous extract of the plant (AEFFt) of respective weights; 0.25 g as well as 0.25 g each of the antacids GESTID, RENNIE, MAALOX, sodium bicarbonate was analyzed for the evaluation of their ANC. GESTID, RENNIE, MAALOX tablets were first crushed in the mortar to obtain a powder before the beginning of the test. Each weighed sample was transferred to a 250 mL beaker and 30 mL of distilled water were added. Then 15 mL of a 1.0 N HCl solution was pipetted and poured into the solution and pH at 1 minute was recorded. The mixture was then stirred constantly for 15 minutes. The pH of the solution was measured and noted. Then the test solution was titrated with an excess of 0.5 N NaOH until a pH of 3.5 was reached. The volume of base consumed was recorded. The mixture was then discarded and the procedure repeated 5 times for every sample.

2.7.2 Determination of the buffer capacity

The buffer capacity was determined according to the recommended method of Holber et al. (39). A quantity of 0.5 g of powder of each sample was put into 25 ml of 0.1N HCl contained in a 50 ml beaker and subjected to constant stirring on the magnetic stirrer. The pH of the mixture was determined at intervals of 0.5, 2, 4, 6.8 and 10 minutes. Then an amount of 5 ml of the mixture was removed using a pipette and replaced with 5 ml of 0.1N HCl. This process was repeated at 10 minute intervals until a pH below 2.75 was attained, which showed that the buffering capacity of the antacid had been exhausted.

2.7.3 Evaluation of the acid-neutralizing speed (ANS)

Evaluation of the acid-neutralizing speed was performed according to the method of Rossett and Rice on samples of the aqueous extract of the plant and certain antacids such as Maalox (aluminum hydroxide and magnesium hydroxide) and sodium bicarbonate. 0.5 g of each sample standardized drug, 0.5 g and 1 g of aqueous extract will be weighed separately and put each in a 250 ml beaker containing 15 ml of 0.1N HCl and 35 ml of distilled water with constant stirring. The electrode PH A will dive into each beaker just after the addition of the test sample. The content 0.1N hydrochloric acid in the burette is continuously added into the beaker at a rate of 2

ml / min. The pH values are recorded every minute for 60 minutes.

2.7.4 Food and drug administration (FDA) trials on antacids

The FDA defines antacids according to the minimum buffering capacity. To be considered an antacid, a molecule must contribute to 25% of the product's total neutralization. We weighed 0.25 g of a sample (plant extract and standard antiulcers), and added 10ml of distilled water and lastly added 2.5 ml of 0.5N HCl and. We homogenized this mixture for 10 minutes on a magnetic stirrer and recorded the pH. For each sample, we repeated the procedure five times. A final pH between 3 and 5 qualified the sample as an antacid, according to FDA.

2.8 In vivo Activity

2.8.1 Preparation of test solutions

2.8.1.1 Preparations of aqueous solution of *Ficus thonningii* fruits extract (AEFFt)

A solution of concentration 50 mg/mL was prepared by dissolving 1500 mg of extract in 30 mL of water thus obtained a 30 mL solution. Then, the mixture was homogenized using a magnetic stirrer. From this solution, we administered to the 2nd, 3rd, 4th and 5th groups of rats according to their respective weights at the following doses: 125 mg/kg (0.5 mL of solution), 250 mg/kg (1 mL of solution) and 500 mg/kg (2 mL of solution).

2.8.1.2 Preparation of reference drugs solutions (Maalox, Omeprazole)

A 20 mL solution of Maalox of concentration 10 mg/mL was prepared by dissolving 200 mg of Maalox in 20 mL of distilled water. Also an omeprazole solution of concentration 4 mg/mL was prepared by dissolving 40 mg of omeprazole in 10 mL distilled water to give a 10 mL solution. The drugs were administered as thus: Omeprazole at 20 mg/kg (1 mL of solution) and Maalox at 100 mg/kg (2 mL of solution).

2.8.1.3 Preparation of ulcerogenic solution

A 30 mL hydrochloric acid/ethanol (HCl/EtOH) ulcer – inducing solution was prepared by mixing 19 mL of 95° alcohol and 0.4 mL HCl then completed the volume to 30 mL.

2.8.2 Anti-ulcer (ulcer-preventive) activity study of AEFFt

30 Wistar rats were used for each of these experiments. The rats were purchased from the FMBS Animal House and left to acclimatize to the laboratory conditions for 5 days, and given free access to water and food. The rats were separated into 6 groups of five animals each.

2.8.3 Induction of ulcers and sacrificing of animals

The animals were separated into 6 groups of 5 animals each and were subjected to a fasting period of 48 hrs but water maintained. One hour prior to administration of the various extract solutions, the water was removed. After 48 hours the first group, the negative control group, received the HCl/EtOH mixture without the extract treatment.

The second, third and fourth groups received specified doses of the aqueous fruit extract, 125 mg/kg (0.5 mL of extract solution), 250 mg/kg (1 mL of extract solution) and 500 mg/Kg (2 mL of extract solution) respectively.

The fifth groups, the positive control groups, received standard or approved drugs, Maalox (Aluminium hydroxide and Magnesium hydroxide) 100 mg/kg and omeprazole 100 mg/Kg respectively.

One hour after oral administration, all animals except those of the first, and fifth groups were administered absolute alcohol, in order to induce gastric ulcers.

Two hours after the HCl/EtOH administration, all animals of that particular group were sacrificed using cervical (neck) dislocation. The stomachs were extracted, opened along the larger curvature and rinsed with a 0.9% NaCl solution. The volume of solution administered (Va) was determined from the following formula [21].

$$Va \text{ (mL)} = \frac{\text{Dose} \left(\frac{\text{mg}}{\text{kg}} \right) \times \text{weight (kg)}}{\text{concentration} \left(\frac{\text{mg}}{\text{mL}} \right)}$$

2.9 Determination of Ulcer Index

All the animals of the treatment groups were sacrificed using cervical (neck) dislocation. Each stomach was ligated to the esophagus and pylorus using sutures and then removed.

The contents of the stomach were collected in the Falcon tubes and centrifuged at 3000 rpm for 15 min. The weight of the mucus corresponding to the centrifugation pellet was weighed using a microbalance (Sartorius: Basic), the volume of the gastric juice of the supernatant was measured by means of a graduated test tube. The pH of each collected gastric juice was measured using a pH meter. Total acidity was determined by titrating the gastric juice with 0.01 N NaOH in the presence of phenolphthalein. The gastric juice was then retained for the determination of pepsin, mucus and total proteins. The stomachs were opened along the large curvature and rinsed with 0.9% sodium chloride (NaCl) solution. Lesions were measured using a graduated scale and the ulcerated surface (US) of each rat was determined. The percentage protection (% P) or the percentage curative ratio (%CR) or percentage inhibition (%I) of the ulcers was determined comparing with the negative control batch [21,26].

$$\%I = \frac{(USc - USt) \times 100}{USc}$$

Where, USc= average ulcer surface area in control

USt= average ulcer surface area in test/treated animals. Or,

$$\text{Percentage protection} = \frac{UI_{\text{control}} - UI_{\text{pretreated}}}{UI_{\text{control}}} \times 100$$

$$\text{Ulcer index (UI)} = \frac{\text{total ulcer score}}{\text{no. of animals ulcerated}}$$

2.10 Statistical Analysis

The results were expressed in terms of mean ± standard deviation. The comparison between the groups was analyzed using one-way analysis of variance, the ANOVA test followed by Turkey's Kramer post hoc test with a significance level at P-value of less than 0.05. The statistical analysis was performed with the aid of GraphPad Inst at version 5.0 software.

3. RESULTS AND DISCUSSION

3.1 Extraction Results

Extraction yield in % =

$$\frac{\text{weight of extract of } F. \text{thomningii} \text{ fruits}}{\text{weight of powdered fruits}} \times 100 = \frac{16.38g}{200g} \times 100 = 8.19\%$$

3.1.1 Phytochemical screening

Qualitative phytochemical analysis of AEFft revealed the presence of total polyphenols, flavonoids, mucilages, saponins, gallic tannins, and betacyanins. The results obtained after screening the extract for secondary metabolites are displayed in Table 1.

3.2 In vitro Antacid Activity

3.2.1 Acid neutralization capacity (ANC) and pH of samples

The various pH values obtained from the mixture: 0.25g of sample +30mL of distilled water +15mL of 1N HCl before back titration with excess of 0.5N NaOH are plotted against the treatment groups in Fig. 1. The bar graph shows the various pH values attained by the samples after stirring before titration. For the negative control, pH of (4.14 ± 0.34); for the plant extract, pH of (8.32 ± 0.33); for sodium bicarbonate, pH of (8.10 ± 0.25); for Gestid (8.16 ± 0.67); for Rennie (8.22 ± 1.39); and for Maalox (7.64 ± 0.54).

From Table 2 below, it was observed that the ANC value for the AEFft = 7.4mEq. The FDA criteria says antacids must have ANC of at least greater than 5mEq. This implies

AEFFt is an antacid according to the ANC test.

3.2.2 Buffer capacity

The pH against time buffering time plot for the AEFft and reference drugs, showed that Maalox exhibited the highest buffering time of 80-90minutes for its pH to drop to below 2.75. The AEFft on the other hand had the least buffering capacity lasting less than 10minutes as shown and for the rest of the reference drugs in Fig. 2. Analysis of the curves gives buffer capacity of less than 10minutes for the AEFft; 70 minutes for the reference drug Rennie; 60minutes for NaHCO₃; 30minutes for Gestid; and 80minutes for Maalox.

3.3 Acid Neutralisation Speed (ANS) (Rosset-Rice test)

The results for the acid neutralization profiles of the AEFft and the reference drugs are displayed in Fig. 3. It was observed that, the plant's whole fruits extract (AEFFt) presented an acid neutralisation speed of 18 minutes; 28 minutes for Rennie; 40 minutes for sodium bicarbonate; 20 minutes for Gestid; and 60 minutes for Maalox. The AEFft brought about faster neutralization effect compared to the control drugs.

Table 1. Presentation of the secondary metabolites in the AEFft

Secondary metabolite	Test or reagents	Results
Mucilage	Absolute ethanol	+
Saponins	Vigorously shaken and allowed to stand for 15 mins	+
	FeCl ₃ 10%	+
Total polyphenols	Lead acetate	+
Flavonoids	Shinoda test	+
	NaOH test	+
Tannins	Cu citrate	+
Catechic tannins	STIASNY	-
Gallic tannins	FeCl ₃	+
	Wagner	-
Alkaloids	Mayer	-
	Hager	-
Anthocyanins	H ₂ SO ₄ + NH ₃	-
Quinones	Conc. H ₂ SO ₄	-
Betacyanins	NaOH and heat for 5 mins	+
Phlobotannins	HCl + heating	-
Steroids	Liebermann -Burchard	-
Resins	Aceticacid + H ₂ SO ₄	-
Oxalates	Glacial aceticacid	-
Cardiac glycosides	Keller-Kiliani	-
Terpenoids	Chloroform + H ₂ SO ₄	-

Key: + present; - absent

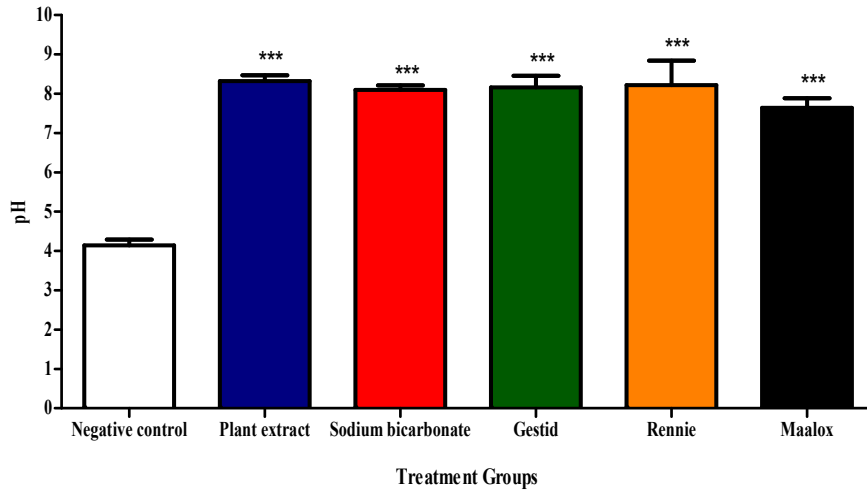


Fig. 1. Effect of plant extract and standard on ANC parameter

Table 2. Data for ANC determination

	AEFFt	Gestid	Maalox	Rennie	NaHCO ₃
Volume of HCl dispensed (mL)	15	15	15	15	15
Volume of NaOH required (mL)	15.2	18.58	8.4	12.7	12.32
Moles of HCl dispensed	15	15	15	15	15
Moles of NaOH required	7.6	9.29	4.2	6.35	6.16
Moles of HCl neutralized (consumed) by antacid in mEq = (N _a - N _b)	7.4	5.71	10.8	8.65	8.84
Neutralizing capacity of antacid per gram (moles HCl neutralized/ g antacid)	29.6	22.84	43.2	34.6	35.36

Molarity of HCl solution = 1N, Molarity of NaOH solution = 0.5N, mass of antacid = 0.25 g

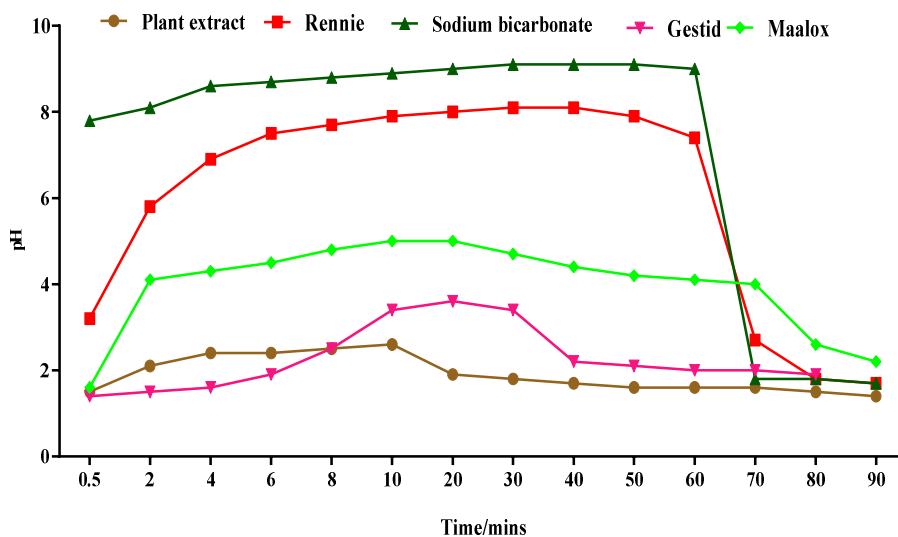


Fig. 2. Effect of plant and drugs reference on buffer capacity

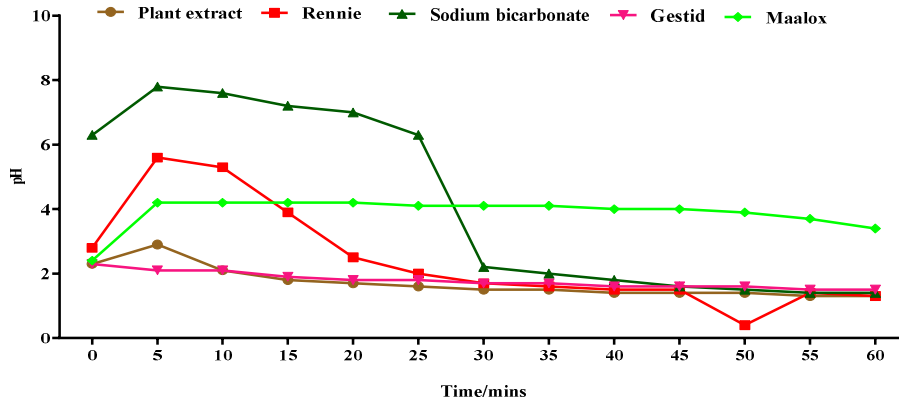


Fig. 3. Effect of plant extract and reference drugs on the Rosset-Rice test

3.4 FDA Test for Antacids

The results of antacid characteristics of the samples by FDA test are shown in Fig. 4. The average pH values of the samples obtained after 50minutes experiment for each sample are: for the negative control = pH of (1,20 ± 0,07 f); plant fruit extract =pH of (4,20 ± 0,10); Gestid = pH of (3,44 ± 0,21); Rennie = pH of (6,98 ± 0,22); sodium bicarbonate = pH of (8,40 ± 0,07); and Maalox = pH of (5,18 ± 0,04). It was observed that the AEFFT qualified as an antacid according to the FDA criteria with average pH = 4.20 ± 0.10 (normal range =3-5).

3.5 In vivo Activity

3.5.1 Anti-ulcer (ulcer-preventive) activity

The anti-ulcer and/or ulcer-preventive activity was characterized by measurement of changes in values of the following parameters: gastric juice pH, gastric juice volume, total acidity of gastric juice, free mucus in gastric juice, and ulcer surface area. The results are expressed in mean ± standard deviation (n = 5). The difference between groups are supposed significant at the *p-value < 0, 05; **p-value < 0, 01; ***p-value < 0,001.

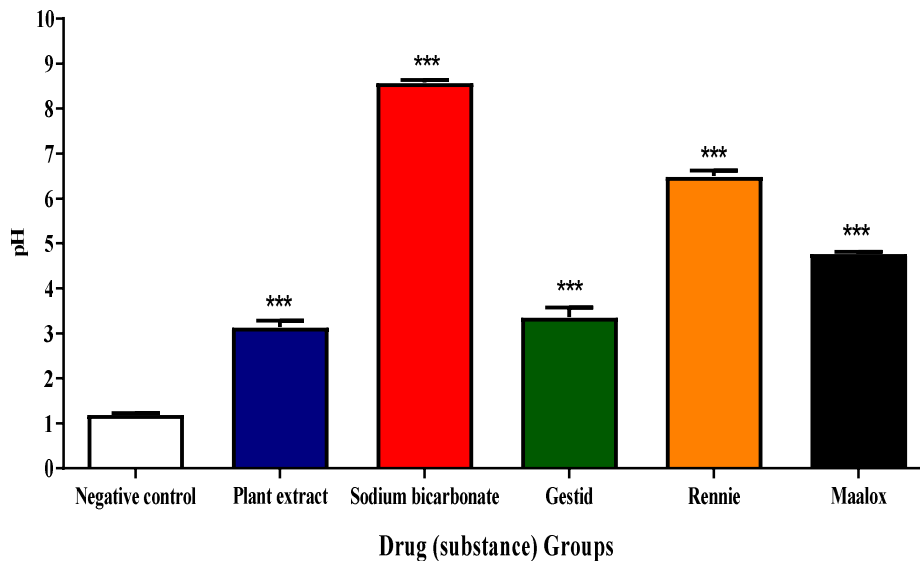


Fig. 4. Effect of plant's fruits extract and standard drugs on FDA

3.5.2 Effect of the plant extract on gastric juice volume

The results of the effect of the plant extract (AEFFt) and other treatment groups are shown in Fig. 5. Results showed a gastric juice production in the negative control of (3.80 ± 1.42) mL, a significant decrease ($p\text{-value} < 0.01$) in gastric juice production following administration of the referenced drugs Omeprazole ($1.13 \pm 0.37^{**}$) mL and Maalox ($1.54 \pm 1.11^*$) mL with $p\text{-value} < 0.05$. For the fruits extract (AEFFt) we observed a significant decrease ($p\text{-value} < 0.01$) in gastric juice production at the doses 125mg/kg and 250mg/kg to be $(1.38 \pm 0.91^{**})$ mL and $(1.39 \pm 0.89^{**})$ mL respectively. At the dose of 500mg/kg, we observed an insignificant decrease ($p\text{-value} > 0.05$) in gastric volume to be (2.03 ± 0.73) mL. It was observed that the plant AEFFt brought about a decrease in free radicals' formation or a decrease inhibition of prostaglandin synthesis which could lead to increased gastric acid secretion.

3.6 Effect of the Plant Extract on Free Mucus

Improved mucus secretory potential of an extract or drug is indicative of their significant role in ulcer healing process. The effects of the AEFFt and other treatment groups are shown in Fig. 6. It was observed that administration of the plant fruit extract and reference drugs brought about an insignificant decrease ($p\text{-value} > 0.05$) in free mucus secretion due to destruction of bound mucus by ulcerogenic substance giving the

following values : for the negative control (1.16 ± 0.38)g, $(0.95 \pm 0,08)$ g for Omeprazole, $(0.86 \pm 0,54)$ g for Maalox, (0.99 ± 0.34) g for the plant extract at the 125 mg/kg dose, $(0.81 \pm 0,49)$ g for the extract at the dose of 250 mg/kg, and $(1.03 \pm 0,82)$ g for the extract at the dose of 500 mg/kg, with $p\text{-value} > 0.05$. Generally, improved mucus secretion suggests ulcer healing. The extract maintained a relatively constant amount of mucus secretion.

3.7 Effect of the Plant Extract (AEFFt) on pH of the Gastric Juice

The pH shows the level of gastric acidity and secretion volume. A low value of gastric pH is an indication of decreased hydrogen ion concentration in gastric juice. This has been linked to pathogenesis of ulcer and gastric damage in experimental animals. The results of the effect of plant extract and other treatment groups on pH are shown in Fig. 7. The pH of gastric juice in the negative control increased insignificantly ($p\text{-value} > 0.05$) to give a value of (3.32 ± 0.95) , similar in omeprazole group to give a value of (3.72 ± 0.55) , and Maalox to give a value of (4.50 ± 1.70) ; we observed contrarily an insignificant decrease ($p\text{-value} > 0.05$) in gastric juice pH by AEFFt at the doses 125 mg/kg to be (2.40 ± 0.57) , 250 mg/kg to be (2.52 ± 0.84) and a significant decrease ($p\text{-value} < 0.05$) in gastric juice pH of the extract at the dose 500 mg/kg to be $(1.66 \pm 0.09^*)$. This was suggestive of the fact that, the extract might not have a net inhibitory effect on gastric acid secretion of rats.

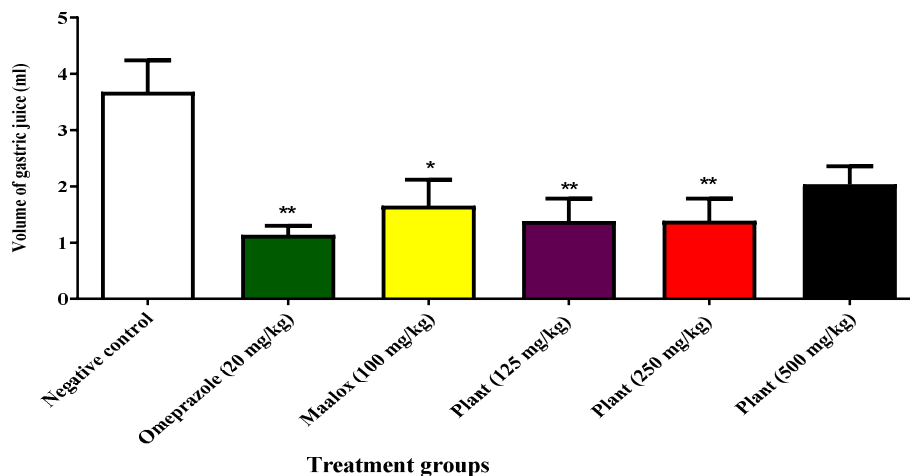


Fig. 5. Effect of plant extract and standard drugs on gastric juice volume

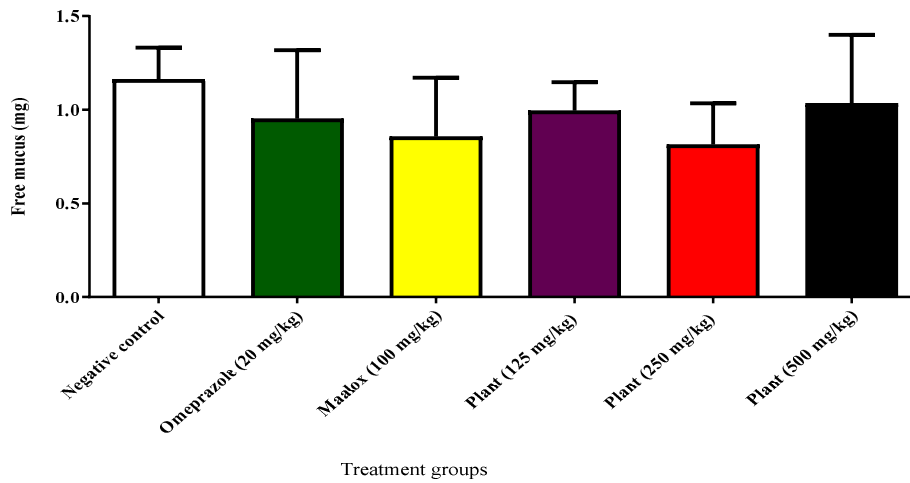


Fig. 6. Effect of plant extract and standard drugs on free mucus in juice

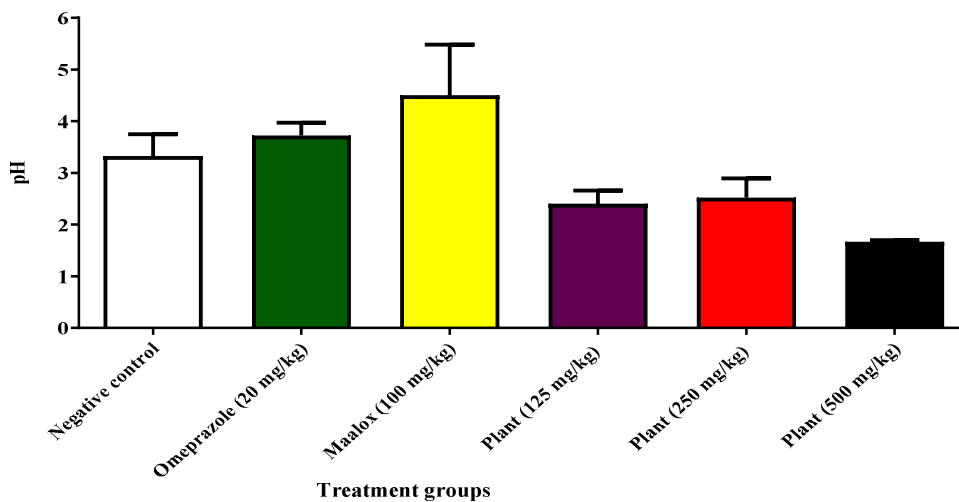


Fig. 7. Effect of AEFft and standard drugs on pH of gastric juice

3.8 Effect of the AEFft on Total Acidity of the Gastric Juice

The results of the effect of AEFft and other treatment groups on total acidity of gastric juice are shown in Fig. 8. Analysis of the results showed an insignificant decrease with $p\text{-value} > 0.05$ of gastric juice total acidity for the negative control to be $(0,01 \pm 0,009)$ mEq, an insignificant decrease with $p\text{-value} > 0.05$ of gastric juice total acidity for the reference groups omeprazole to be $(0,008 \pm 0,001)$ mEq and Maalox $(0,002 \pm 0,001)$ mEq, an insignificant increase with $p\text{-value} > 0.05$ in gastric juice total

acidity for the fruits extract at the dose 125 mg/kg to be $(0,02 \pm 0,007)$ mEq and at 250 mg/kg to be $(0,02 \pm 0,006)$ mEq and a significant increase with $p\text{-value} < 0.05$ of gastric juice total acidity at the dose 500 mg/kg to be $(0,04 \pm 0,004)$ mEq.

3.9 Effect of the AEFft on Ulcers Surface

The results of the effect of AEFft and other treatment group on ulcer surface area are shown in Fig. 9. The results showed a significant decrease ($p\text{-value} < 0.001$) in the ulcer surface area following administration of the test fruits extract and reference drugs.

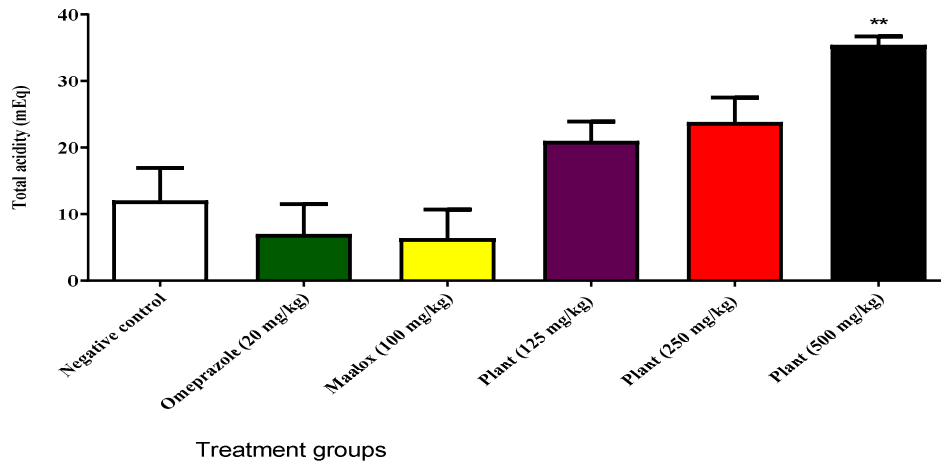


Fig. 8. Effect of AEFft and standard drugs on total acidity of gastric juice

The ulcerated surface area for the negative control group was $(17.65 \pm 4,31) \text{ mm}^2$, for omeprazole is $(1.18 \pm 0,16^{***}) \text{ mm}^2$ giving a percentage protection of 93.31%, ulcerated surface area for Maalox is $(0,21 \pm 0,02^{***}) \text{ mm}^2$ giving a percentage protection of 98,81%, ulcerated surface area in the 125 mg/kg group is $(5,16 \pm 0,57^{***}) \text{ mm}^2$ giving a percentage protection of 70,76%, ulcerated surface area for the 250 mg/kg group is $(4,95 \pm 0,47^{***}) \text{ mm}^2$ giving a percentage protection of 71,95%", and

ulcerated surface area for the 500 mg/kg group is $(0,72 \pm 0,05^{***}) \text{ mm}^2$ giving a percentage protection of 95,92%.

3.9.1 Histology of organs exposed to treatment

In Fig. 10 is the histology of the dissected stomachs of the rats showing the effect of the different treatments received.

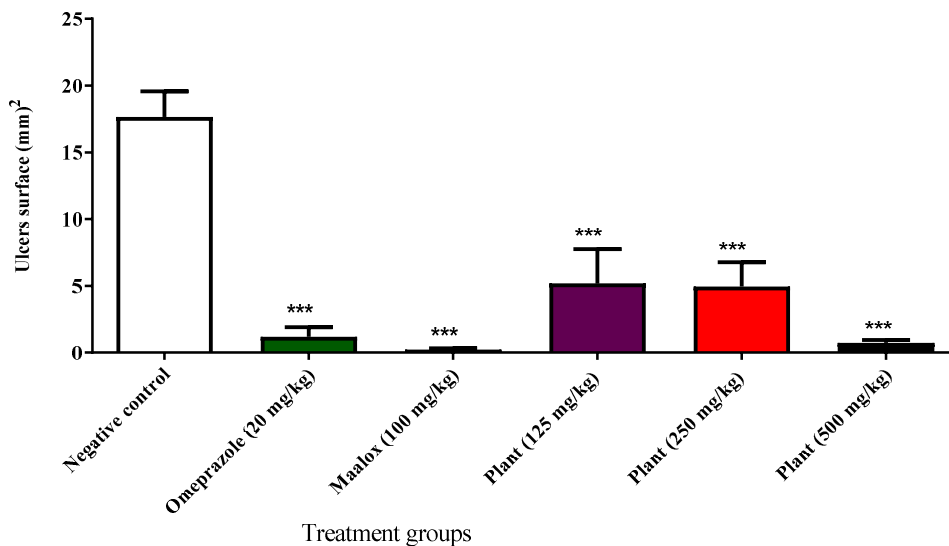
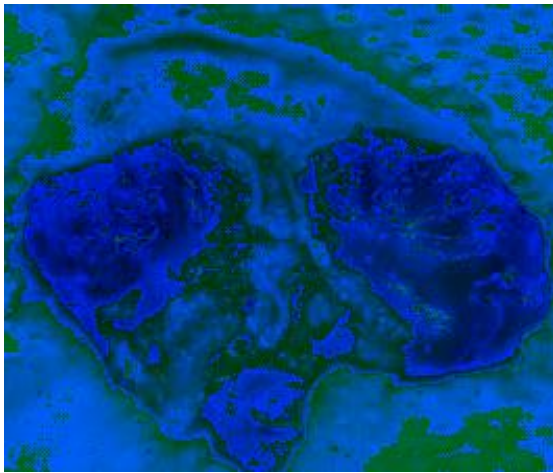
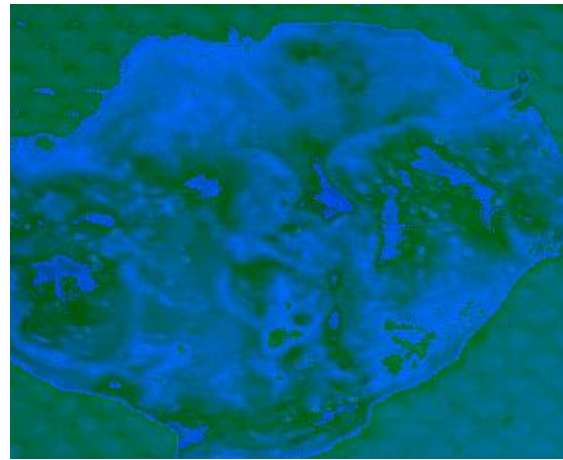


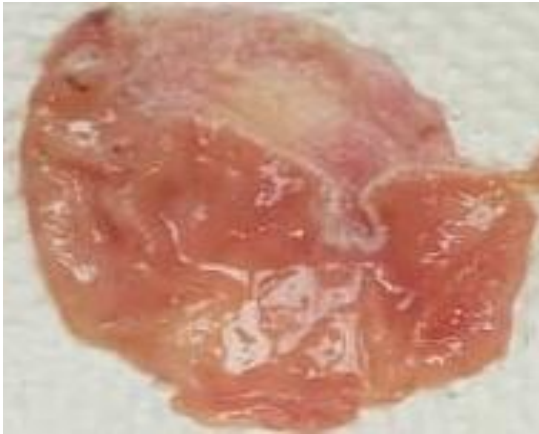
Fig. 9. Effect of AEFft and standard drugs on ulcers surface



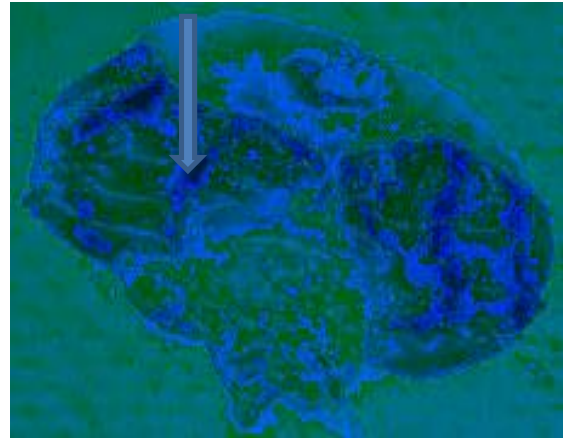
Negative control (ulcerogenic solution)



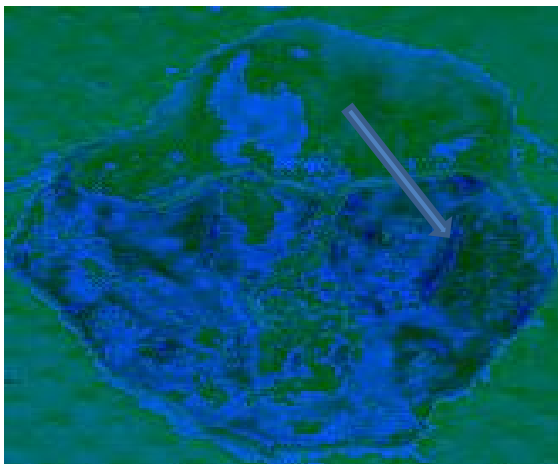
Maalox (100 mg/kg)



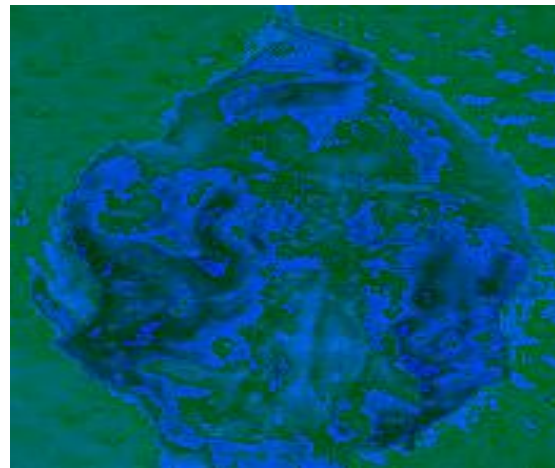
Omeprazole (20 mg/kg)



Plant extract (AEFFt) 125 mg/kg



Plant extract (AEFFt) 250 mg/kg



Plant extract (AEFFt) 500 mg/kg

Fig. 10. Images showing the dissected stomachs of various animal groups pretreated with AEFFt, before inducing ulcers with HCl/EtOH solution

4. DISCUSSION

The genus *Ficus* englobes plants of the Moraceae family and is one of the most populous in number of species of all plant genera with over 800 different species [27]. Of these species, 60 are found in Cameroon according to Berg et al. [28,29]. A wide pool of bioactive phytochemicals including tannins, flavonoids, alkaloids, terpenoids and phenolic glycosides have been reported to be responsible for the observed gastroprotective and antiulcerogenic properties of the various plants used in PUD management [3]. With the urge to describe such bioactive phytochemicals in medicinal plants of Cameroon, we investigated the effects of an aqueous extract of the fruits of *Ficus thonningii* Blume collected from the West region of Cameroon on hydrochloric acid/ethanol (HCl/EtOH) –induced peptic ulcers. HCl and EtOH both stimulate acid secretion and accelerate gastric mucosal necrosis and apoptosis by damaging gastric mucosal defense system [30]. HCl/EtOH-induced gastric damage was observed in the gastric mucosa as elongated black, red lines parallel to the long axis of the stomach of the rats. Ethanol readily penetrates the gastric mucosa due to its ability to solubilize the protective mucus and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin, causing damage to the membrane. Moreover, alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, through disruption of the vascular endothelium and facilitating vascular permeability; it also increases activity of xanthine oxidase [31].

The results of the phytochemical screening of the aqueous extract of whole fruits of *Ficus thonningii* revealed presence of the following bioactive phytochemicals: total polyphenols, mucilage, saponins, flavonoids, gallic tannins, and betacyans. These results corroborate to those obtained with work done on leaves of *Ficus thonningii* in 2015 by Omoregie et al. [7], in 2014 by Dangarembizi et al. [8], (who obtained flavonoids, saponins, phlobatannins, and anthraquinones in both aqueous and methanolic extracts while terpenoids and alkaloids were detected in methanolic extracts only); and with work done on stem bark of *Ficus thonningii* in 2010 by Usman et al. [23], and still on the stem bark in 2019 by Fokunang et al. [32], (saponins, quinones, coumarins, catechic tannins, phlobotanins, anthocyanin, polyphenols, flavonoids and betacyanes with a hydro-

ethanolic extract). The minor differences in results of secondary metabolites obtained could be attributed to the use of different solvents for extraction. Phytochemicals are obtained in higher concentrations of these phyto-chemicals in methanolic extracts than there were in the aqueous extracts [8]. This was probably because methanol had a higher polarity index than water hence could extract a higher concentration of phenolic compounds. In addition, most plant secondary metabolites are organic in nature and hence partition well in organic solvents. This justifies the use of palm wine as a solvent in ethno-medicinal systems of West Africa according to Akinsulire et al. in 2007 [8].

The four different *in vitro* tests carried out to investigate the antacid properties of our plant's fruits extract gave varying results. The FDA test gave a final average pH of 4.20 ± 0.10 confirming it an antacid according to the FDA (normal range being 3–5). The acid neutralization capacity (ANC) of AEFfT gave a value of 7.4mEq also corroborating with FDA criteria for ANC of antacids that says antacids must have an ANC of at least 5mEq. The extract however had a poor buffering capacity lasting only 10 minutes and a weak acid neutralization profile compared to the controls.

The anti-ulcer (ulcer- preventive) activity of the aqueous extract of fruits of *F. thonningii* (AEFFt) was evaluated by studying the changes in gastric juice volume, free mucus in gastric juice, gastric juice pH, total acidity, and ulcer surface area values. The stomach protects itself from acid damage by the secretion of a mucous layer that helped to protect gastric epithelial cells by trapping secreted bicarbonate at the cell surface [18]. Gastric mucus was soluble when secreted but quickly formed an insoluble gel that coated the mucosal surface of the stomach, slowed ion diffusion, and prevented mucosal damage by macromolecules such as pepsin. Mucus production is stimulated by PGs E_2 and I_2 , which also directly inhibit gastric acid secretion by parietal cells. Ethanol treatment caused induction of oxidative stress intracellularly and led to transition of mitochondrial permeability and depolarization, which further led to cell death in the gastric mucosa [33]. The results showed gastroprotective activity of the AEFfT as observed with the decrease in gastric juice volume and significant decrease (p -value <0.001) in ulcer surface area with a percentage protection of 95.92% in the 500 mg/kg group of rats which was due to the antioxidant, antiulcer,

and antisecretory properties of the different bioactive phytochemicals qualified through screening. The antioxidant activity demonstrated was due to presence of flavonoids as also shown by work done on figs and roots of *F. thonningii* in 2014 by Fongang et al. [29]. Flavonoids also showed antiulcer effect by exerting antioxidant, anti-secretory, anti-inflammatory and mucosa regenerative activities as has also been shown in work done on *Piper umbellatum* in 2016 by Junior et al. [3]. Saponins with antioxidant properties also exhibited *in vivo* antiulcer activity which corroborated with the result obtained from the work done on leaves of *Bauhinia purpurea* in 2014 by Paguigan et al. [34]. Mucilages found in the AEFft also showed gastroprotective effect which corroborated with the work done on *Bryophyllum pinnatum* in 2014 by Sharma et al. [33].

5. CONCLUSION

At the end of this study, it was demonstrated that, the aqueous extract of fruits of *F thonningii* (AEFFt), contained the bioactive phytochemicals total polyphenols, flavonoids, tannins (gallic), mucilages, saponins, and betacyanins most of which have dose-dependent antiulcer and /or antioxidant activity on HCl/EtOH –induced gastric ulcers in wistar rats. The extract showed *in vitro* antacid activity by FDA criteria. The AEFft showed the highest gastroprotective activity at a dose of 500 mg/kg.

The overall findings in this study clearly stress that plant products represent a rich source of bioactive molecules with antiulcer potential. Moving from traditional uses to preclinical studies the efficacy of certain herbal remedies has been substantially investigated by *in vitro* and even *in vivo* studies, and, in some cases, their activity has been ascribed to specific classes of phytochemicals such as alkaloids, tannins, simple phenols and polyphenols (particularly flavonoids). The use of this plant as a category 1 improved traditional medicine in peptic ulcers management was confirmed by preclinical antiulcer activity demonstrated in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was taken from the institutional review board of the Faculty of Medicine

and Biomedical Sciences. Authorization was obtained from the head of the laboratory of preclinical animal studies and toxicology research of the faculty of medicine and biomedical sciences (FMBS), of the University of Yaoundé I.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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