



# Antiulcer Effect of an Aqueous Extract of *Syzygium aromaticum* (Myrtaceae) Buds in Wistar Rats

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**Abstract:** The buds of *Syzygium aromaticum* (Myrtaceae) is used in traditional medicine in the treatment of gastric ulcer. This pathology affects approximately 10% of the world's population. The current study aims to verify the antiulcer effect of an aqueous extract of the buds of *Syzygium aromaticum* (EASA) in wistar rats. For this, the 1000 mg/kg B.W. dose of EASA was tested on gastric ulcer induced with (Eth/HCl/H<sub>2</sub>O) in rats compared to the effect of 20 mg/kg B.W. of Omeprazole for 14 days. Also, a phytochemical screening was carried out on this extract. After 3 days of ulcer induction, the ulceration index was 3 and the ulceration percentage was 100% in the stomachs of the rats. The results after 14 days of treatment with EASA show that the ulceration index and the percentage of ulceration are 0.5 and 8.83% against 0 for Omeprazole. The aqueous extract of *Syzygium aromaticum* therefore treats the induced ulcer at 91.17% against 100% for Omeprazole. These results are confirmed by observations of scars on histological sections on the fourteenth day. Phytochemical screening indicates that EASA contains sterols, polyterpenes and polyphenols, quinone compounds, alkaloids and gallic tannins. These results would justify the use of *Syzygium aromaticum* buds in traditional medicine in the treatment of gastric ulcer.

**Keywords:** Phytochemical Screening, *Syzygium aromaticum*, Ulcer, Induction, Omeprazole

## 1. Introduction

An ulcer is defined as a loss of substance in the gastric wall reaching deep into the muscle tissue [1]. Ulcer is one of the most widespread gastrointestinal disorders, affecting approximately 10% of the world's population, particularly populations in non-industrialized countries [2]. To this end, studies have shown that this pathology represents almost 31.65% of cases of consultation in gastroenterology departments [3]. These causes are multiple, however, many studies have shown that alcohol causes the degradation of the gastric mucosa and consequently induces intense lesions that penetrate the submucosa, increasing the infiltration of neutrophils, which then delays the healing process of

ulcerated gastric tissue [4].

Many plants are used in the treatment of gastric ulcer by people. Indeed, in Africa, 80% of the population use medicinal plants for their health care [5]. For this reason, the WHO recommends that scientists establish scientific bases for plants used in traditional medicine. *Syzygium aromaticum*, also called clove, is an aromatic tree whose buds have analgesic, major anti-infective, anti-inflammatory, antioxidant properties and are used in the treatment of gastric ulcer [6].

The objective of this study is to verify the anti-ulcer effect of an aqueous extract of *Syzygium aromaticum* buds in rats by determining the ulceration index, the percentage of ulceration and the percentage of healing. But also, to determine the major chemical groups in this extract responsible for its effects.

## 2. Materials and Methods

### 2.1. Material

#### 2.1.1. Plant Material

The plant material used for this study consists of dried buds of *Syzygium aromaticum* (Myrtaceae). These buds were purchased at the Adjamé market, a town in the center of the city of Abidjan (Ivory Coast), in April 2022 and identified at the Floristic National Center (FNC) of the Felix Houphouët-Boigny University in comparison to herbarium number 15261.

#### 2.1.2. Animal Material

For the study of the antiulcer effect of the aqueous extract of buds of *Syzygium aromaticum*, rats of the wistar strain of the species *Rattus norvegicus* (Muridae) weighing between 180 and 200 grams are used.

These animals are bred in the animal facility of the UFR Biosciences of the Felix Houphouët-Boigny University (Abidjan, Ivory Coast), and fed, ad libitum, with pellets produced by Ivograin® (Abidjan) for the breeding of rodents.

This study is conducted in accordance with the European directives of November 24, 1986 (86/609/EEC) and decree of April 19, 1988 [7] on animal experiments in research.

#### 2.1.3. Chemical and Pharmacological Substances

The chemical substances used are Ethanol (Gayomart, France), hydrochloric acid (Kuhlmann, France). The pharmacological substance used is Omeprazole (Advacare Pharma, United States).

### 2.2. Methods

#### 2.2.1. Preparation of the Aqueous Extract of *Syzygium aromaticum* Buds

The dried buds of *Syzygium aromaticum* are crushed, the powder obtained is used for the extraction, 100 g of this powder are macerated in 1 L of distilled water for 24 hours. The solution obtained is filtered once, three times on absorbent cotton and finally twice on Watthman No. 4 paper. The filtrate is dried in an oven at 50°C. for 3 days. The aqueous extract of *Syzygium aromaticum* buds (EASA) obtained is brown in color.

#### 2.2.2. Phytochemical Study of the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae)

This study, which is carried out according to Abo [8] qualitative methods described. The aqueous extract of buds of *Syzygium aromaticum* (EASA) is used for this test. Its purpose is to highlight the major chemical groups of pharmacological interest, namely sterols and polyterpenes, flavonoids, tannins, quinone compounds, saponosides, polyphenols and alkaloids.

The tests are carried out by reactions in tubes.

#### 2.2.3. Study of the Curative Activity of the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae)

##### (i). Preparation of the Ulcerogenic Solution

The ulcerogenic substance is prepared according to Kamigui [9] the method described. In a beaker, 100 mL of a

mixture will be prepared with the proportions of the substances below.

- 1) Ethanol: 60% of the mixture, either 60 mL.
- 2) Hydrochloric acid (HCl): 1.7% of the mixture, either 1.7 mL.
- 3) Distilled water: 38.3% of the mixture, i.e. 38.3 mL.

This mixture (Ethanol / HCl / H<sub>2</sub>O), which constitutes the ulcerogenic solution, is homogenized using a magnetic stirrer before its administration to the animals.

##### (ii). Test of the Curative Activity of the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae)

This study is carried out in rats and consists of inducing gastric ulcer for 3 (three) days and treating the ulcers for 14 (fourteen) days [10].

##### (iii). Composition of Batches and Treatment of Gastric Ulcer

In this study, 48 rats are divided into 4 groups of 12 rats, placed in cages until the end of the experiment. Each batch is subdivided into 3 sub-batches such that one (1) first sub-batch is sacrificed after the three (3) days of oral induction at a daily dose of 8 mL/kg B.W. with the ulcerogenic substance (D0). The two (2) other sub-batches are sacrificed on the seventh (D7) and the fourteenth day (D14) of administration of the substances tested. These animals are subjected to a water diet for 24 hours before the first administration of the ulcerogenic solution, and remained so until 6 hours after the second administration of this substance on the second day, before being treated or not with the aqueous extract of *Syzygium aromaticum* buds or with Omeprazole. Batch 1 or negative control (TN) received only 1 mL of distilled water per rat (1 mL/rat) during the fourteen (14) days of experimentation. Then, the ulcer is induced to the rest of the animals for three days. From D0, batch 2 or positive control (PT) received distilled water until D14. Batch 3 received 1000 mg/kg B.W. of the aqueous extract of *Syzygium aromaticum*. Finally, batch 4 received Omeprazole which is the reference substance at 20 mg/kg B.W.

Distilled water, aqueous extract of *Syzygium aromaticum* buds or Omeprazole are administered by gavage for 14 days.

The body weight of rats in subsets 3; 6; 9 and 12 belonging respectively to the Negative Control (TN), Positive Control (TP), EASA and Omeprazole batches is measured, on D0 then on the 7th and 14th days.

##### (iv). Assessment of Healing

Seven (7) days after the last administration of this ulcerogenic solution (D7), the rats of the second (2nd) subgroup 1, 2, 3 and 4 are sacrificed. Those of the third (3rd) sub-lots 1, 2, 3 and 4 are also sacrificed at the end of the experiments (D14). Just after having sacrificed them, the stomachs of the rats are removed, opened, washed and their mucous membranes are observed with a magnifying glass to evaluate the various healings.

On examination, the following will be sought: an irritated gastric mucosa, hemorrhagic points and furrows, non-hemorrhagic points and furrows. Only furrows and hemorrhagic points are considered as ulcerations [11]. Each

stomach is rated from 0 to 3, depending on the number of ulcerations:

0 = no ulcerations, 1 = 1 to 2 ulcerations, 2 = 3 to 4 ulcerations;

The ulceration index is calculated according to the following formula:

$$UI = \frac{\text{Somme des cotations} \times \text{Percentage of ulcered stomach}}{\text{Animal number}}$$

UI: Ulceration Index

The percentage of ulceration is calculated according to the following formula:

$$PU = \frac{IU}{3} \times 100\%$$

PU: Percentage of ulceration

The percentage of healing of the gastric mucosa induced by the plant or by Omeprazole according to the following formula:

$$PH = \frac{IU (\text{Positive control}) - IU (\text{tested substance})}{IU (\text{Positive control})} \times 100$$

PC: Percentage of healing.

UI: Ulceration Index

#### (v). Histological Study

After macroscopic observation of the stomachs, histological sections are made on these organs. The histology technique made it possible to obtain thin sections of the stomachs.

Observing these cuts will make it possible to detect the depth of the ulcerations and the cellular level of healing. Thus, the organs are fixed in 10% formalin for 48 hours at room temperature and sections are made with a microtome.

Sections are analyzed using a tri-ocular electron microscope (Olympus CX31, Philippines) surmounted by a camera (AmScope, MD130) connected to a computer (HP EliteBook Folio 1040, China).

### 2.3. Processing of Results

The computer program GraphPad *Prism* version 5.01 (San Diego CA USA) is used for the statistical analysis of the results. The results are processed by analysis of variance (ANOVA), followed by Dunnett's multiple comparison test. The difference between two values is considered significant for  $P < 0.05$ . This software was used for the statistical processing of the curative effect of the aqueous extract of *Syzygium aromaticum* buds.

GraphPad *Prism* software version 5.01 (San Diego CA USA) was used to plot the graphs of the antiulcer effect of the aqueous extract of *Syzygium aromaticum* buds.

## 3. Results

### 3.1. Phytochemical Screening of the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae)

The results of the tests carried out by tube reactions on the aqueous extract of buds of *Syzygium aromaticum* are given in

Table 1.

The screening carried out on the aqueous extract of *Syzygium aromaticum* buds reveals the presence of sterols, polyterpenes and polyphenols, quinone compounds, alkaloids and gallic tannins. However, we note the absence of flavonoids, saponosides and catechic tannins.

**Table 1.** Chemical composition of the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae).

Compounds		Test or reagent	Results
Sterols and polyterpenes		Liebermann	+
Polyphenols		ferric chloride	+
Flavonoids		Cyanidine	-
Saponins		vigorous shaking	-
quinone compounds		Borntraeger	+
		Dragendorff	+
Alkaloid		Bouchardat	+
Tannins	Catechic	Stiasny	-
	Gallic	hydrochloric acid	+

(+): Presence of the compound

(-): Absence of the compound

### 3.2. Curative Effect of the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae) on Gastric Ulcer Induced in Wistar Rats

#### 3.2.1. Macroscopic Observation

Examination of the stomach of the negative control rats (TN) shows that, on the 7<sup>th</sup> day (D7) and the 14<sup>th</sup> day (D14), the gastric mucosa shows almost no ulcerative points and furrows "Figure 1".

However, on D0, on the stomachs of the rats of the other groups sacrificed, observations were made. Thus, these stomachs present gastric lesions localized in the mucosal part of the stomach and represented by ulcerations, redness of the mucosa, edemas, hemorrhages, erosions and generalized perforations "Figure 1".

On the 7th day of the treatment with EASA and Omeprazole, the stomachs of the rats which were sacrificed the hemorrhagic zones have partly disappeared. On the other hand, in the positive control rats (TP) sacrificed, there is always at the level of the mucosa, edemas, hemorrhages, erosions and perforations.

On day 14, on the stomach of rats treated with the aqueous extract of buds of *Syzygium aromaticum*, only a few hemorrhagic points are observed. Also, in rats treated with Omeprazole, all ulcerations completely disappeared on the gastric mucosa. On the other hand, the positive control batch always shows ulcerations "Figure 1".

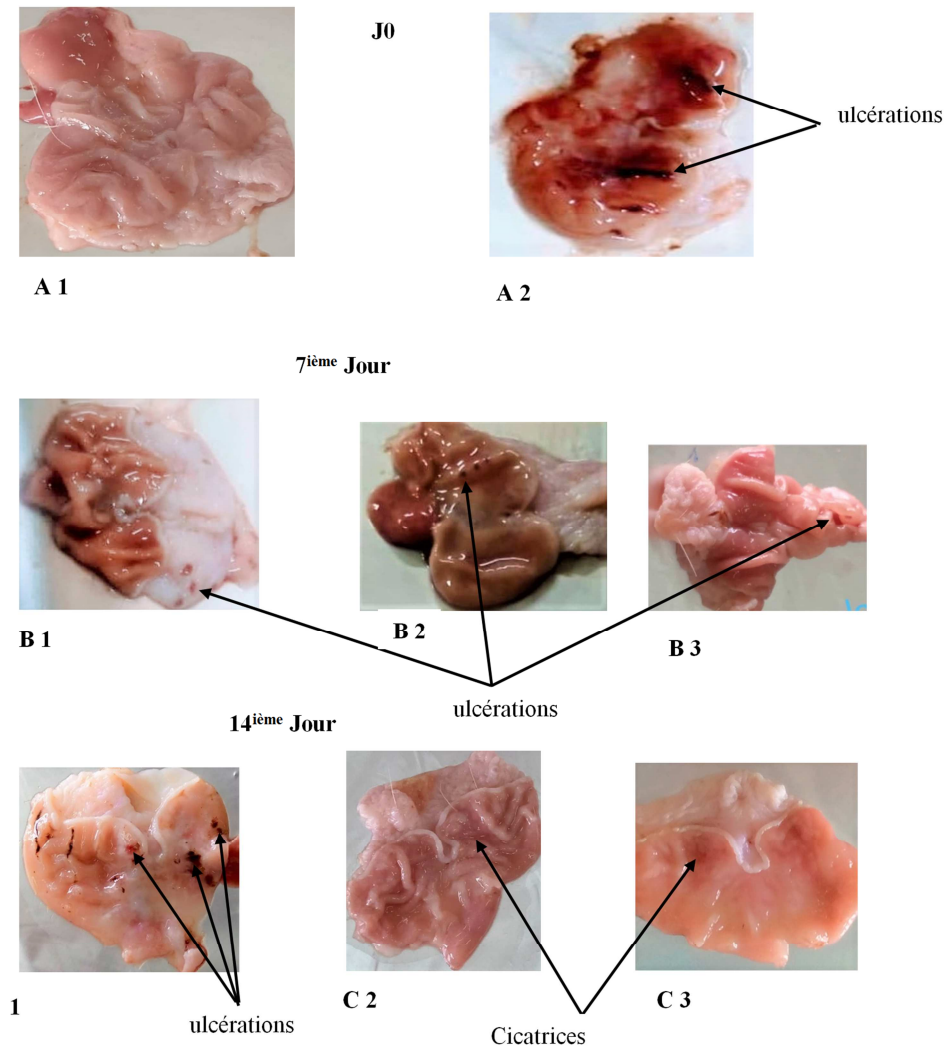
#### 3.2.2. Microscopic Observation

The macroscopic results were confirmed by histological sections. In fact, the observations of histological sections made from the stomachs show that the untreated rats, that is to say having received only distilled water, have a normal mucosa.

Also, the rats having received the ulcerogenic mixture revealed the existence of ulcerative phenomena sitting at the level of the epithelium.

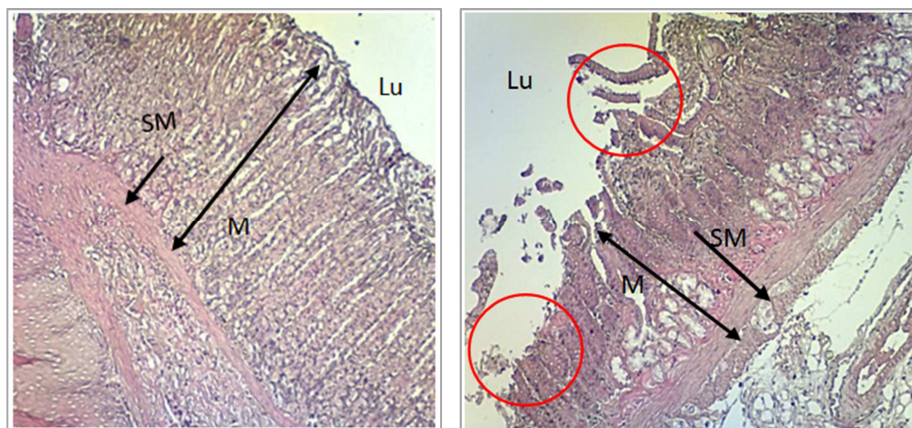
The stomachs of rats treated with the aqueous extract EASA and Omeprazole after 14 days of treatment, show phenomena of repair of the lesion. All parts of the necrotic submucosa

have disappeared and the mucosa is practically regenerated “Figures 2 and 3”.

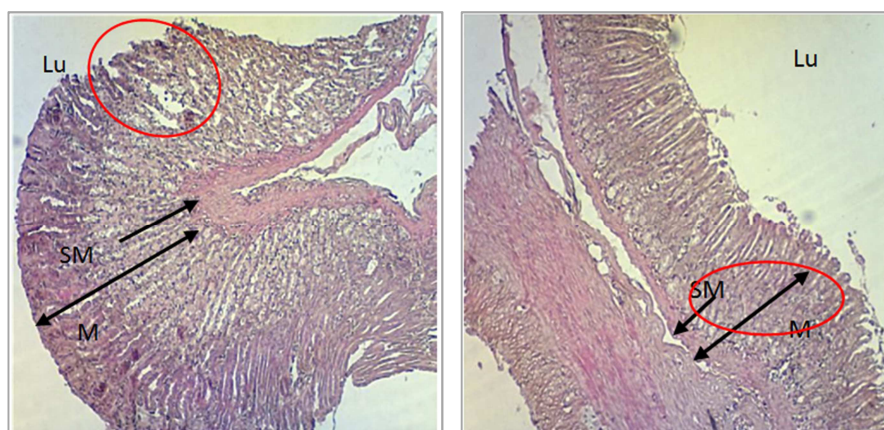


**Figure 1.** Photographies des estomacs des rats wistar rendus ulcéreux, puis traités ou pas avec l'extrait aqueux de bourgeons de *Syzygium aromaticum* (Myrtaceae) ou l'Oméprazole.

J0: Jour 0; A1: Photographie d'estomac de rats négatif au jour 0; A2: Photographie d'estomac de rat témoin positif au jour 0; B1: Photographie d'estomac de rats positif au jour 7; B2: Photographie d'estomac de rats traités avec EASA à la dose de 1000 mg/kg P.C. au jour 7; B3: Photographie d'estomac de rats traités avec l'Oméprazole à 20 mg/kg P.C.; C1: Photographie d'estomac de rats positif au jour 14; C2: Photographie d'estomac de rats traités avec EASA à 1000 mg/kg P.C au jour 14; C3: Photographie d'estomac de rats traités avec l'Oméprazole à 20 mg / kg P.C au jour 14.



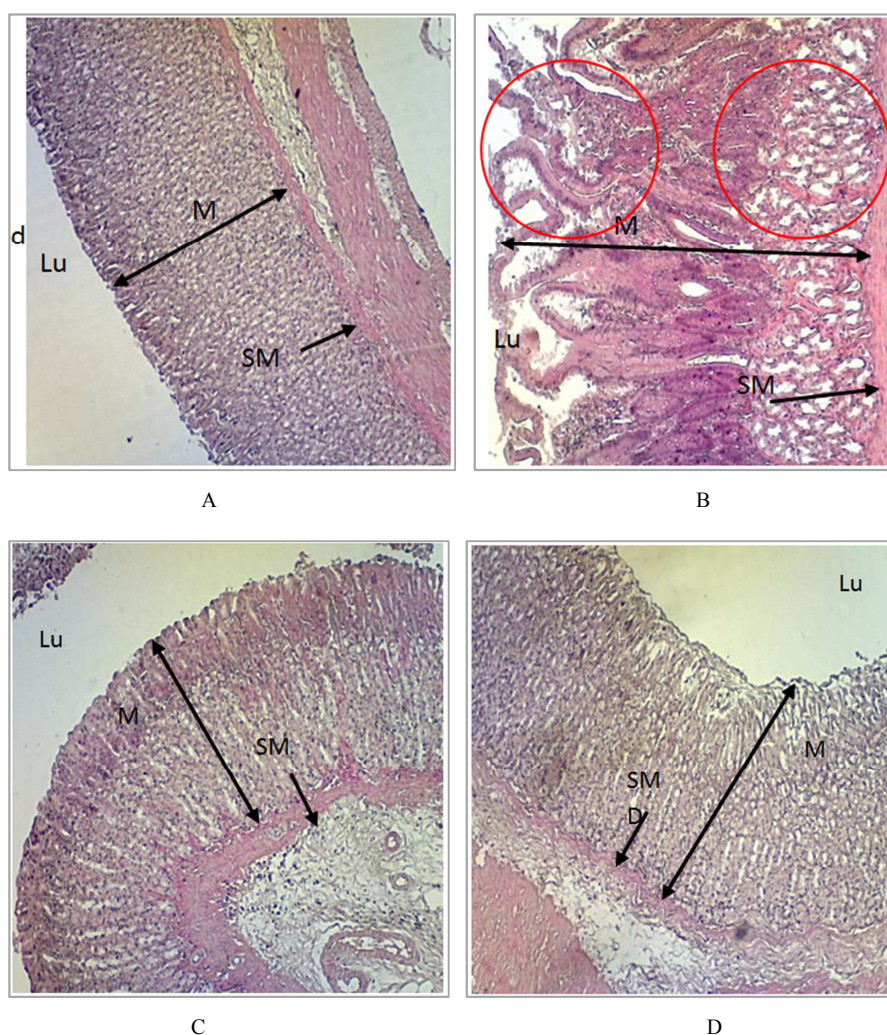




**Figure 2.** Micrographs of the stomachs of rats treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) or Omeprazole after ulcer induction on the 7<sup>th</sup> day.

Staining: Eosin-hematoxylin; Magnification: X40

A: Negative control; B: Positive control; C: Aqueous extract of *Syzygium aromaticum* at 1000 mg/kg B.W.; D: Omeprazole at 20 mg/kg BW; M: Mucosa; SM: Sub mucosa; Lu: Light



**Figure 3.** Micrographs of the stomachs of rats treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) at a dose of 1000 mg / kg B.W or Omeprazole after ulcer induction on the 14<sup>th</sup> day.

Staining: Eosin-hematoxylin; Magnification: X40

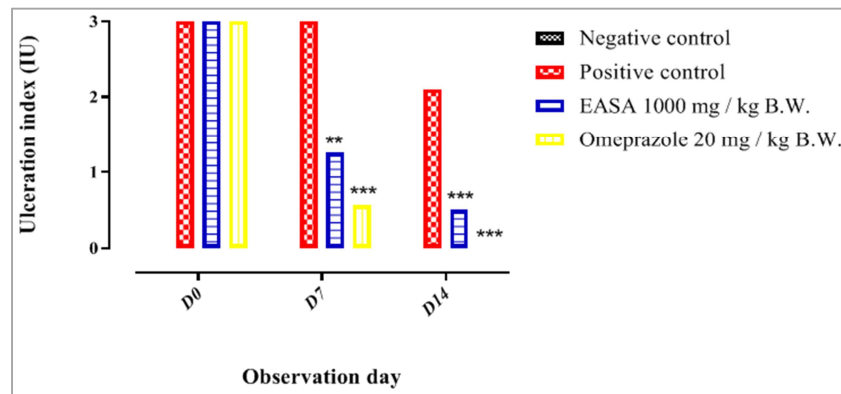
A: Negative control; B: Positive control; C: Aqueous extract of *Syzygium aromaticum* at 1000 mg/kg B.W.; D: Omeprazole 20 mg/kg B.W. M: Mucosa; SM: Sub mucosa; Lu: Light

### 3.2.3. Effect of the Aqueous Bud Extract of *Syzygium aromaticum* (Myrtaceae) and Omeprazole on the Ulceration Index

The ulceration index obtained after three 3 days of induction is shown in “Figure 4”. Examination of the ulceration index shows that on D0, that is to say after the 3 days of induction of the ulcer, the UI is equal to 3 for all the

rats having received the ulcerogenic substance.

While on the 7<sup>th</sup> day of the experiment, the ulceration index of the rats treated with the dose of 1000 mg / kg B.W. or 20 mg / kg B.W. of omeprazole decreases significantly ( $P < 0.001$ ) compared to its value on D0 for each batch. That of the positive control batch remains unchanged. The values are respectively 1.25; 0.56 and 3 for EASA, Omeprazole and positive control.



\*\* :  $P < 0,01$ ; \*\*\* :  $P < 0,001$ ;  $n = 4$

**Figure 4.** Variation of the ulceration index in rats rendered ulcerated and treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) or Omeprazole.

On D14, the UI is zero in the batch treated with Omeprazole and 0.5 in the batch that received the aqueous extract of *Syzygium aromaticum* buds.

These values show a significant decrease ( $P < 0.001$ ) compared to their UI on D0. For the positive control group whose UI is 2.1, the variation is not significant ( $P > 0.05$ ).

During the treatment period, the UI of the wistar rats of the negative control batch, i.e. treated with distilled water, remained zero.

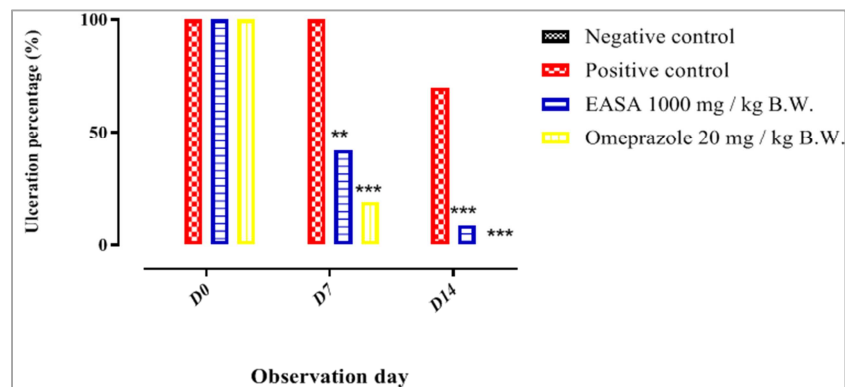
### 3.2.4. Evaluation of the Percentage of Ulceration of Wistar Rats Treated with the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae) and Omeprazole

Over the 14 days of experimentation, the percentage of ulceration of the negative control group remained nil. On the other hand, on D0, the percentage of ulceration determined for

the rats treated with the ulcerogenic substance is 100%.

On the 7<sup>th</sup> day, in rats having received the aqueous extract of buds of *Syzygium aromaticum* or Omeprazole, we observe a significant reduction ( $P < 0.001$ ) in the percentage of ulceration compared to the value on D0 in these batches. At this date the PU in the positive control batch remains intact. The percentage of ulceration is 42% for EASA, 19% for Omeprazole against 100% for the Positive Control batch.

On D14, the percentage of ulceration is 0 in the Omeprazole batch and 8.83% for the batch that received EASA (1000 mg/kg B.W.). There is almost a significant disappearance ( $P < 0.001$ ) of the furrows and ulcerous point in these batches. In the positive control, the decrease in PU is not significant ( $P > 0.05$ ) with a value of 70% “Figure 5”.



\*\* :  $P < 0,01$ ; \*\*\* :  $P < 0,001$ ;  $n = 4$

**Figure 5.** Variation in the percentage of ulceration in rats rendered ulcerated and treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) or Omeprazole.

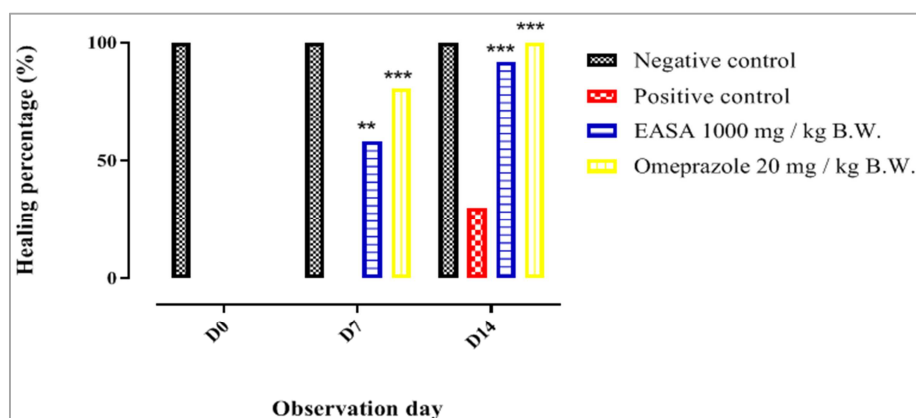
### 3.2.5. Evaluation of the Percentage of Healing in Wistar Rats Treated with the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae) and Omeprazole

Observation of the negative control rats (TN) shows that, on the 7<sup>th</sup> day (D7) and the 14<sup>th</sup> day (D14), the gastric mucosa shows almost no ulcerative points and furrows.

On D7, we observe a healing percentage of 58% with the dose of 1000 mg / kg B.W. of EASA and 81% with Omeprazole.

While at the 14<sup>th</sup> day, the ulcer induced on D0 had practically healed on the stomachs of rats treated with the aqueous extract of *Syzygium aromaticum* buds and Omeprazole. The healing percentage in these batches is 91.17% and 100% respectively.

“Figure 6” shows the variations in the percentage of healing observed in each stomach during the 14 days of treatment after induction of the ulcer.



\*\* :  $P < 0,01$ ; \*\*\* :  $P < 0,001$ ;  $n = 4$

**Figure 6.** Variation in the percentage of healing in rats rendered ulcerated and treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) or Omeprazole.

### 3.2.6. Evaluation of the Body Weight of Rats Treated with the Aqueous Extract of Buds of *Syzygium aromaticum* (Myrtaceae) and Omeprazole

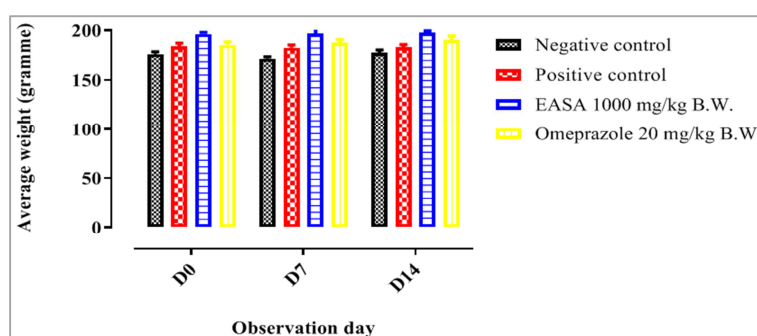
The body weight of sub-batches 3, 6, 9 and 12 were taken on D0, D7 and D14. The results show that the weights of the animals did not vary significantly ( $P > 0.05$ ) on D7 and D14 compared to their value on D0 in each sub-batch.

Indeed, the weight of the rats in subgroups 3, 6, 9 and 12 on

D0 were respectively  $176 \pm 2.3$ ;  $183.8 \pm 3.1$ ;  $196 \pm 1.6$  and  $185 \pm 3.3$ g.

On D14, after weighing the animals of sub-batches 3, 6, 9 and 12, the weights obtained were respectively  $177.1 \pm 3$ ;  $183 \pm 2.5$ ;  $197.3 \pm 2.1$  and  $190.2 \pm 4$ g.

“Figure 7” presents the variations in the body weight of the rats over the 14 days of treatment.



$P > 0,05$ ;  $n = 4$

**Figure 7.** Variation in body weight of rats rendered ulcerated and treated with the aqueous extract of buds of *Syzygium aromaticum* (Myrtaceae) or Omeprazole.

## 4. Discussion

This study is carried out with the aim of verifying the curative effect of the aqueous extract of *Syzygium aromaticum* buds on gastric ulcer induced in rats.

The curative effects of EASA and those of Omeprazole, which is the reference anti-ulcer substance, were evaluated on induced gastric ulcer in wistar rats. After the three days of induction of the ulcer the Ulceration Index was equal three (3) in all the batches having received the ulcerogenic substance. Authors obtained the maximum ulcerations from the second



day of administration of the mixture while [12] obtained it after 3 days. According to Lambert studies, the action of the Ethanol / HCl / Water mixture would be essentially necrotic [13]. Thus, its oral administration leads to intense erosive gastritis rapidly progressing to necrosis. According to Nyilimana [10], prolonged fasting and stress related to animal maintenance conditions are also ulcerogenic factors. Also, in our study, after 3 days of treatment with the ulcerogenic substance, we obtained 100% as the percentage of ulceration for the fasting animals having received it. Histologically, the lesions reach the submucosa and the muscle with inflammatory cells in the fundic gland cells. Nyilimana observed these effects of the Ethanol / HCl / Water mixture [10].

Treatment of gastric ulcerations with EASA at a dose of 1000 mg/kg B.W., resulted in 91,17% healing of gastric lesions after 14 days. With Omeprazole used at a dose of 20 mg/kg B.W., 100% healing is obtained. In other words, at the EASA level we have a significant antiulcer activity ( $P < 0.001$ ), just like the reference antiulcer. Omeprazole inhibits the gastric enzyme  $H^+ / K^+ - ATPase$  (the proton pump), which catalyzes the exchange of  $H^+$  and  $K^+$  ions. Similarly, Omeprazole causes effective inhibition of basal acid secretion and food-stimulated acid secretion [14]. Therefore, EASA like Omeprazole could directly inhibit the gastric enzyme  $H^+ / K^+ ATPase$ . Also, the extract could exert an inhibition of gastric acid secretions like Omeprazole. Indeed, Omeprazole exerts an anti-secretory action by coupling to the histamine receptor [15-16]. From a histological point of view, the extract leads to total healing of the muscle tissue, of the sub-mucosa. Also, the inflammatory cells disappeared. However, some points of lesions persist at the level of the surface epithelium.

The screening carried out on the aqueous extract of *Syzygium aromaticum* reveals the presence of sterols, polyterpenes and polyphenols, quinone compounds, alkaloids and gallic tannins. These secondary metabolites would be at the origin of the pharmacological effects of the aqueous extract of buds of *Syzygium aromaticum* (EASA).

Indeed, tannins are recognized as powerful local healing agents [17]. The presence of tannins explains the antiulcer effect of several natural products such as *Arctostaphylos uva-ursi*, and *Viburnum pulus* (Caprifoliaceae). These chemical components enhance tissue repair. In addition, they have antioxidant activity [18]. Furthermore, polyphenols also have antioxidant activity [19]. As EASA contains tannins and polyphenols, we can hypothesize that its healing activity is due to one or more molecules of these compounds. Indeed, according to Tossou [20], alkaloids, polyphenols and tannins have an antioxidant power that promotes tissue regeneration. Finally, sterols and terpenes give plants antipyretic, analgesic and anti-inflammatory properties [21]. These compounds present in EASA, could have enabled it to significantly reduce inflammation due to ulcerations.

The effect of the aqueous extract of *Syzygium aromaticum* buds was verified on the body weight of wistar rats during the 14 days of experimentation. This study shows that the body weight of the rats does not vary significantly from day 0 to day

14 of treatment with EASA. The aqueous extract of *Syzygium Aromaticum* buds therefore had no significant effect on the body weight of wistar rats.

## 5. Conclusion

The study of the anti-ulcer effect of the aqueous extract of the buds of *Syzygium aromaticum* reveals that this extract promotes the healing of ulcerative lesions. The histological analysis of the stomachs of rats treated with the extract of the plant, shows that the regeneration of most of the cells destroyed during the induction of the ulcer with a normal aspect of the mucosa. These actions would be linked to the presence of alkaloids, tannins, sterols of polyphenols present in the aqueous extract of the buds of this plant. These results would therefore justify the use of *Syzygium aromaticum* buds in traditional medicine in the treatment of gastric ulcer.

## Conflict of Interest

The authors declare that they have no competing interests.

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