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PRODUCTION OF AN ANTI-GASTRIC ULCER SYRUP FROM *KHAYA GRANDIFOLIOLA* BARK.

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ABSTRACT: Gastric ulcers (GU) represent a world prevalence of 5-10% and a 10.4% prevalence in Cameroon. The frequent use of non-steroidal anti-inflammatory drugs (NSAIDs) is one of the major causes. The effective treatments available are expensive and have serious side effects. Treating GU with medicinal plants could be a safer alternative solution. In this work, we formulated an anti-ulcer syrup using the aqueous extract of *Khaya grandifoliola* bark (KG). GU was induced in rats by administering orally indomethacin at 5mg/kg for 5 days. A treatment for 5 days with *Khaya grandifoliola* syrup (12.4 mg/kg), *Spathodea campanulata* syrup (200 mg/kg) and misoprostol (100 µg/kg), a reference pharmaceutical drug was given. KG syrup quality control test gave a pH of 5.18 and a density of 1.28. This syrup caused a 79.72% reduction of ulceration surface area and a significant increase in mucus mass of 85.29% ($p < 0.05$) compared to the Ulcer control. Biochemical parameters revealed a significant increase in antioxidants (65.38% GSH; 45.65% Catalase at $p < 0.05$) and a decrease of 76.87% in malondialdehyde after indomethacin induction. The formulated KG syrup exhibited therapeutic anti-ulcer effects and could be used as a natural alternative to treat GU.

INTRODUCTION: Gastric ulcers represent a major health problem. It has a world prevalence of 5-10% and a 10.4% prevalence in Cameroon, affecting both young and aged persons. Gastric ulcers result from an imbalance between some aggressive (e.g., *Helicobacter pylori*, overproduction of acid and pepsin) and defensive factors (e.g., increased blood flow and mucus, prostaglandins production) in the stomach, which penetrates the muscular layers, causes acute lesions and irritations in the gastric mucosa^{1,2}.

The long-term use of NSAIDs is one of the principal risk factor for gastric ulcer formation, with an estimated prevalence of 15%–30% chronic NSAID consumers experiencing significant gastrointestinal side effects³. Their main mechanism of injury is the inhibition of the two cyclooxygenase enzymes, cyclo-oxygenase-1 (COX-1) and cyclo-oxygenase-2 (COX-2), thereby reducing the production of prostaglandins and enhancing mucosal damage. A decrease in mucosal blood flow and increase in free radicals being released also lead to the formation of ulcers⁴.

For the treatment of these ulcers, pharmacological medications used include: antibiotics agents like clarithromycin and amoxicillin, proton pump inhibitors (PPIs) like omeprazole, histamine receptor antagonists (H2 receptor antagonists) like Ranitidine, antacids, prostaglandin analogues, and

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mucosal protective agents⁵. However, the use of these medications has led to many adverse effects such as headaches, diarrhea, abdominal pain, nausea, sleep deprivation, pneumonia, osteoporosis-related fractures, hypersensitivity, inflammation of the kidneys, iron and vitamin B12 deficiency. Thus, the use of alternative therapeutic strategies with known natural medicinal plants, having diversified properties in treating GU is needed. In Cameroon, stem barks of *Khaya grandifoliola* are used by traditional physicians against gastric ulcers⁶. It possesses the following beneficial activities: anti-ulcer, anti-microbial, anti-inflammatory and anti-oxidant activity⁷. *Khaya grandifoliola* belongs to the family Meliaceae popularly known as the mahogany family⁸. It is found in Southern, Central and Western Africa. This plant is rich in active biomolecules and present low toxicity effects⁹.

Previous studies indicated that *Khaya grandifoliola* extract improved gastric mucosal defence, reduced gastric lesions on mucosal walls and gastric acidity in rats¹⁰. A minimal inhibitory concentration (MIC) of 12.4 mg/kg body weight gave a 100% inhibition of gastric ulcers in stress-ulcerated rats which is the safe therapeutic dose with high efficacy and no toxicity¹¹. Also, the crude extract and the main fractions of *Spathodea campanulata* bark have shown the capacity to inhibit *H. pylori* urease in a heterologous bacterial model and treat gastric ulcer¹²⁻¹⁴.

Based on those studies, we aimed at producing an anti-ulcer syrup using the aqueous extract from the bark of *Khaya grandifoliola* and *Spathodea campanulata*. Comparisons are made with the effects of misoprostol, a reference pharmaceutical drug. The syrup effects were determined using an experimental animal model of gastric ulcer.

MATERIAL AND METHODS:

Plant Material: The stem bark of *Khaya grandifoliola* and *Spathodea campanulata* were obtained from Fouban, Noun division in the west region of Cameroon on the 24th of January 2025. Those stem barks were dried at room temperature, ground at a mill then bagged, labelled and stored in a refrigerator at 4°C before use. The identification of the plants using the leaves and grains was done at the National Herbarium of Cameroon in

Yaoundé, where the specimens were deposited under respective reference number 23434 YA and 50085/HNC.

Animal Material: The experimental animals were 24 Wistar rats of male sex (150 g-250 g) procured from the animal house of the Faculty of Science, University of Yaounde I. The animals were placed on normal laboratory diet and water. All the experimental protocols were submitted to and approved by the Ethical Clearance Reference N°BTC-JIRB2023-083 of the Joint Institutional Review Board for Animal and Human Bioethics (JIRB), Cameroon.

Preparation of the Plant Extract: The aqueous extract was prepared by decoction of 10g of the plant powder (size <600µm) in 600 mL of distilled water for 20 minutes. The resulting mixture was filtered using Whatman paper N°4. The filtrate obtained was concentrated in an oven (50°C) until a dry extract was obtained.

Syrup Preparation: Syrups were formulated as described by Kouoh *et al.*¹⁵. Briefly 250 mL of the filtrate obtained from decoction was mixed with 500g of brown sugar plus 10mL of lemon juice. The mixture after homogenisation was kept on the heat until caramelization begins. After cooling, the syrup was packaged in 80 mL bottles.

Gastric Ulcer Induction: Indomethacin (a NSAID) was administered orally at dose 5mg/kg for 5 days. A single group of 4 rats (ulcer control) were sacrificed under ether 24 h after the last administration of indomethacin while the rest were subdivided into five groups of four rats each which received treatment once a day for 5 days consecutively as follows:

Group 1 (Normal control): 1mL of distilled water.

Group 2 (Negative control): 5mg/kg body weight of indomethacin + 100 mL of distilled water + 180g of sugar

Group 3 (Positive control): 5mg/kg body weight of indomethacin + 100µg/kg Misoprostol

Groups 4: 5mg/kg body weight of indomethacin + 200mg/kg body weight of the syrup of *Spathodea campanulata*

Group 5: 5mg/kg body weight of indomethacin + 12.4mg/kg body weight of the *Khaya grandifoliola*.

Twenty-four hours after the last administration, all the rats were killed under ether. Morphological observation of the ulcers and ulcer indices were determined¹⁶. The rate of healing of the ulcers in each experiment was calculated by comparison of the ulcer index of rats treated with those of untreated rats. Additionally, the stomachs of normal rats were also used for comparison. The ulcerated stomach portion of each rat were divided into two parts of which one was homogenized to perform oxidative stress parameters analysis and the other was set in 10% buffered formalin awaiting histological studies.

Histopathological Examination: The stomach stored in formaldehyde (10%) were dehydrated using upgraded alcohol series and cleared with xylene before being embedded in molten paraffin blocks using standard methods. Sections of the stomach wall were made at the regions perpendicular to the surface of each ulcer lesion with those of normal stomachs which were used for comparison. Eosin and haematoxylin were used to stain the stomach sections. The stained sections were observed in the microscope and microphotographs were recorded.

Evaluation of gross Mucosal Damage and Mucus Mass: The stomach of each rat was cleaned to get rid of stomach content and blood clots. The cleaned stomachs were pinned and stretched on a corkboard, and pictures of the stomach mucosa's overall structures were taken, for proper observation and documentation.

The area of ulceration was measured and calculated according to the methodology outlined by Tan *et al.*¹⁷ Using a microscope cover slide, the mucus covering the glandular part of the stomach of each rat was gently scraped and carefully weighed using a sensitive electronic balance.

In-vivo Antioxidant Activity and Nitric Oxide (NO) Assay: Briefly, 1g of the stomach of each animal was crushed and homogenized in tris-HCl buffer (50mM) to obtain a 20% (w/v) solution. After centrifugation at 5000rpm for 10minutes, supernatants were collected and stored at -20°C awaiting dosage of some oxidative stress markers.

Malondialdehyde (MDA) concentration was measured as described by Wilbur *et al.*¹⁸ Catalase (CAT) activity was measured according to protocols of Sinha.¹⁹ Reduced glutathione (GSH) levels were measured as described by Ellaman *et al.*²⁰ The nitrite content was quantified by measuring nitrite concentration using the Griess reagent assay as described by Fermor *et al.*²¹

Statistical Analysis: Data was analyzed with GraphPad prism version 8.0.2 and results expressed as mean \pm standard deviation. Multiple comparisons were done following ordinary one-way ANOVA and then Tukey post hoc test to determine significant differences between groups. p value < 0.05 was considered statistically significant.

RESULTS AND DISCUSSION: *Khaya grandifoliola* syrup was formulated from aqueous extract of the bark with pH and density in accordance with European Pharmacopoeia values (rajouter la reference): **Table 1**. This syrup caused a 80% reduction of ulceration surface area and a significant increase in mucus mass of 85% (p < 0.05) compared to the ulcer control: **Table 2**. These findings align with those of Njikam *et al.*,¹¹ who reported a minimal inhibitory concentration of 12.4mg/kg body weight, demonstrating a 100% inhibition of gastric ulcers induced by acid and stress. Biochemical analysis revealed a significant increase in antioxidant levels, with 65.38% in GSH and 45.65% in Catalase at p< 0.05. A decrease of 76.87% in pro-oxidants (MDA) due to indomethacin induction.

TABLE 1: PHYSICOCHEMICAL PARAMETERS OF THE SYRUP

Parameter	Result	Pharmacopoeia Value (EU)
pH	5.18	4-7
Density	1.28	1.26-1.32

The macroscopic aspect of the rats' stomachs in **Fig. 1**, shows slight decrease of ulcer lesions in the negative control (C) compare to the ulcer control (B). However, with treated groups (D, E and F) the decrease is more important. For histological sections in **Fig. 2**, the normal control revealed a well-structured stomach microarchitecture, while the ulcer and negative controls displayed desquamation of the mucosal epithelium and inflammation in the muscularis layer.

However, desquamation, inflammation and the size of stomach membrane were restored in the animals receiving the syrups (at doses 200mg/kg, and 12.4mg/kg respectively for *Spathodea campatulata* and *Khaya grandifoliola*) as well as in the positive control (Misoprostol 100µg/mL). *Khaya grandifoliola* syrup may reduce gastric ulcer surfaces by increasing pH, mucus production and anti-oxidative status. This is in accordance with the study of Khalid S. A²¹⁻²⁴. In our study, we used misoprostol as the reference drug. It is a synthetic analogue of prostaglandin E1; it has a dual action

in gastroenterology: antisecretory by reducing the production of gastric acid (mainly hydrochloric acid, produced by the stomach) and protective of the gastric mucosa by stimulating the production of mucus and bicarbonates²⁵. Based on the results obtained, we can compare the effects of the two formulated syrups to those of Misoprostol. Thus, the three tested medications are ranked in descending order as follows **Table 2**: the syrups (phytomedicines) of *Khaya grandifoliola* and *Spathodea campatulata*, then misoprostol (conventional medication).

TABLE 2: PHYSIOLOGICAL PARAMETERS OF ULCERATION

Treatment	Surface ulceration (mm ²)	% of Ulceration	Mucus production (mg)	pH of gastric juice
Normal	-	-	320±2.00 ^a	3.90
Indomethacin	4.79±0.09	100	134±4.00 ^b	4.10 ^a
Negative control	2.91±0.02	61.13	154±2.00 ^b	5.50
Misoprostol	1.20±0.00 ^a	10.29	175,3±0.57 ^c	5.90 ^a
<i>Khaya grandifoliola</i> syrup	0.49±0.01 ^a	12.39	248,3±2.08 ^c	6.50 ^a
<i>Spathodea campatulata</i> syrup	0.59±0.00 ^a	25.21	208±4.00 ^c	6.10 ^a

The values in the table represent the mean ± STD (Standard deviation), n=4. ^aSignificantly different from the Negative control at p < 0.05. ^bSignificantly different from the normal control at p < 0.05. ^cSignificantly different from the Negative and normal control at p < 0.05.

TABLE 3: BIOCHEMICAL PARAMETERS OF ULCERATION

Treatment	Catalase x10 ⁻³ (µm/min/g organ)	GSH (mmol/g organ)	MDA (mol/g organ)	NO (µM/g organ)
Normal	6.6±0.43 ^a	0.55±0.03 ^a	1.55±0,007 ^a	4.69±0.25 ^a
Indomethacin	4.6±0.93 ^b	0.26±0.01 ^b	9.08±0,096 ^c	1.75±0.01 ^b
Negative control	5.1±0.10 ^b	0.31±0.006 ^b	6.97±0,054 ^b	3.06±0.03 ^b
Misoprostol	6.9±0.10 ^c	0.42±0.005 ^c	3.04±0,15 ^c	7.25±0.11 ^c
<i>Khaya grandifoliola</i> syrup	6.7±0.91 ^c	0.43 ±0.003 ^c	2.10±0.18 ^c	10.0±0.12 ^c
<i>Spathodea campatulata</i> syrup	6.8±0.93 ^c	0.41±0.001 ^c	2.26±0.036 ^c	7.58±0.04 ^c

The values in the table represent the mean ± STD (Standard deviation), n=4. ^aSignificantly different from the Negative control at p < 0.05. ^bSignificantly different from the normal control at p < 0.05. ^cSignificantly different from the Negative and normal control at p < 0.05.

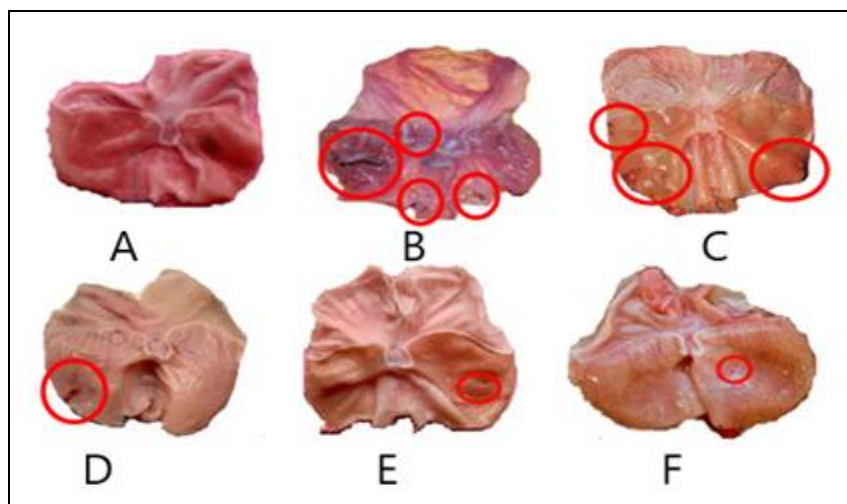


FIG. 1: MACROSCOPIC IMAGE OF NON-ULCERATED AND ULCERATED STOMACHS. (A): Normal control; (B): Ulcercontrol; (C): Negative control, (D): after Misoprostol (reference drug administrated at 100µg/ml); (E): after *Spathodea campatulata* at dose 200 mg/kg bw; (F): after *Khaya grandifoliola* at dose 12.4 mg/kg bw. The circles indicate ulcers.

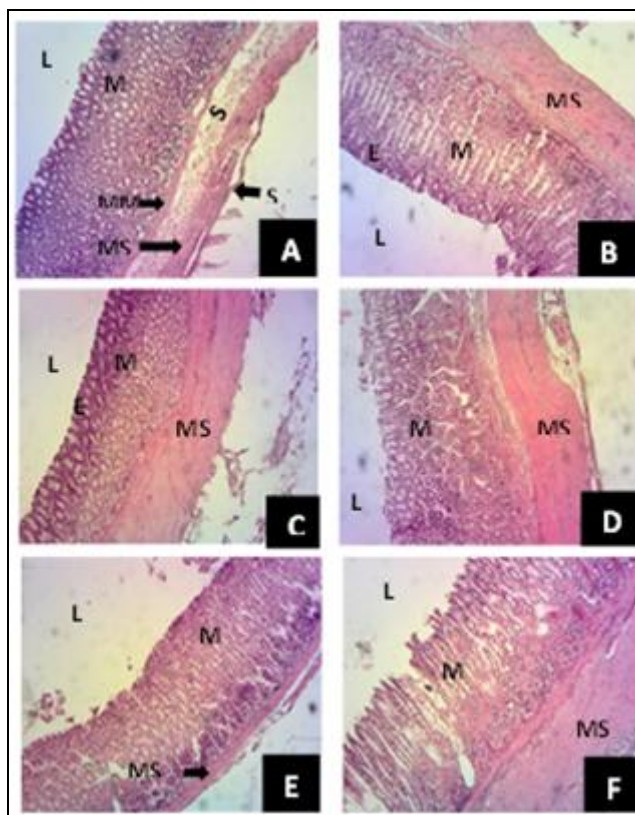


FIG. 2: HISTOLOGICAL SECTIONS OF NON-ULCERATED AND ULCERATED STOMACHS (HE, 40X). A) Normal control that underwent no intoxication and received distilled water, B) Transverse control, C) animals that underwent intoxication and received distilled water, D) Positive control that underwent intoxication and received a commercial reference formulation at dose 100 μ g/ml, E and F = test groups that underwent intoxication and received the formulations of *Spathodea* (200mg/kg), *Khaya* syrup (12.4mg/kg). L = lumen, M = mucosa, MM = muscularis mucosa, MS = muscularis, SM = submucosa, S = serosa, E = erosion.

CONCLUSION: The syrup from *Khaya grandifoliola* bark exhibits therapeutic anti-ulcer effects compared to Misoprostol or *Spathodea campatulata* syrup. This indication may be a scientific support to the practice of traditional physicians. Especially in contexts where, the high cost and limited access to conventional medications face significant challenges.

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