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Anti-ulcer Properties and Microbiological, Physicochemical, and Phytochemical Quality of a Revealed Medicinal Recipe

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Abstract For centuries, various pathologies have been treated with traditional medicines derived from medicinal plants. Such remedies must meet three requirements: safety, therapeutic efficacy, and quality. The objective of this study was to assess the anti-ulcer effect, microbiological and physicochemical quality, and phytochemical profile of a revealed medicinal recipe (RMR). Two models of gastroduodenal ulcer induction using two ulcerogenic agents were employed to evaluate the anti-ulcer effect in laboratory rodents. Classical methods were applied for microbiological and physicochemical quality assessment, following standardized AFNOR procedures, while phytochemical profiling was carried out using tube coloration reactions. The results showed that the recipe protects the gastric mucosa against ulcers induced by hydrochloric acid–ethanol mixture and ethanol in the presence of diclofenac. The recipe was free of pathogenic flora (Salmonella and *Staphylococcus aureus*) as well as total and fecal coliforms. The contamination levels of total aerobic mesophilic flora were 2.2×10^6 CFU/mL and 10^4 CFU/mL for yeasts and molds, compared with the standards of 10^3 and 10^2 CFU/mL, respectively. Moreover, the pH, electrical conductivity, total dissolved solids, and solvent content were 4.61, 3.625 mS/cm, 1835 ppm, and 20.04%, respectively. Phytochemical screening revealed the presence of tannins, alkaloids, flavonoids, anthraquinones, oses and holosides, mucilages, reducing sugars, and the absence of saponosides. In conclusion, the revealed medicinal recipe is a mucosal protector of good microbiological and physicochemical quality and is rich in secondary metabolites.

Keywords: *Ulcers, microbiological quality, phytochemistry, revealed medicinal recipe*

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1. Introduction

Medicinal recipes are empirical preparations generally composed of natural products, particularly medicinal plants. They have been used for generations to treat a wide range of ailments. Such recipes often arise from ethnobotanical knowledge, relying on the synergistic association of several plant ingredients [1]. Many medicinal recipes, including revealed medicinal recipes, are used in Congolese traditional medicine to relieve or treat various diseases. Among these is the revealed medicinal recipe (RMR), developed in a religious setting to treat multiple pathologies, although its composition has not been disclosed by its promoter. A preliminary study of the gastrotoxicity of RMR in subacute treatment in normal rats was deemed necessary. Therefore, the objective of the present study was to evaluate the anti-ulcer properties, microbiological and physicochemical quality, and chemical composition of RMR.

2. Methodology

Presentation of the RMR

The recipe used was a yellowish liquid preparation with a strong fuel-like odor. When left to stand, it separated into two distinct phases: aqueous and non-aqueous.

2.1. Experimental Animals

The experimental animals consisted of male Swiss albino mice weighing between 15 and 30 g, and male Wistar rats weighing between 150 and 200 g. All animals, aged approximately 2 to 3 months, were maintained under standard conditions, subjected to a light/dark cycle, at an ambient temperature of 27 ± 1°C, with free access to food and tap water.

2.2. Evaluation of the Effect of the Recipe on the Gastroduodenal Mucosa

2.2.1. Evaluation of the Effect of RMR on the Gastric Mucosa in Normal Laboratory Rodents Without Induced Ulcers

The gastric mucosa, covered by mucus and bicarbonates, constitutes a natural protective barrier against acidity and aggressive agents. Any administered substance may either preserve or alter this integrity. It is therefore necessary to first evaluate the effect of the medicinal recipe on the normal gastric mucosa, both acutely and subacutely, prior to any experimental ulcer induction.

2.2.2. Acute Model in Mice

Adult albino mice were fasted for 18 hours prior to experimentation and randomly divided into three groups of five animals each as follows:

- Group 1 (control): received only distilled water at 5 mL/kg;
- Groups 2 and 3: received RMR at 0.23 mL/kg and 1.15 mL/kg, respectively.

Sixty minutes after administration, the mice were euthanized according to ethical procedures (cervical dislocation). The stomachs were quickly excised by laparotomy, ligated, and opened along the greater curvature. After gentle rinsing with saline solution (0.9% NaCl) to remove gastric contents, each stomach was spread on a white background and examined macroscopically.

2.2.3. Subacute Model in Rats

Male Wistar rats were treated by daily gavage for a period of 14 days, divided into three groups of three animals each, and treated according to the following protocol:

- Group 1: received only distilled water at 5 mL/kg;
- Groups 2 and 3: received RMR at 0.23 mL/kg and 1.15 mL/kg, respectively.

Twelve hours prior to sacrifice, the animals were subjected to water fasting. After euthanasia, the stomach of each animal was excised and opened along the greater curvature. The excised stomachs were gently rinsed with physiological water for observation.

2.3. Evaluation of the Effect of RMR on Induced Gastroduodenal Ulcers in Laboratory Rodents

2.3.1. Effect of RMR on Induced Gastric Ulcers in Mice

The method reported by Elion Itou and *al*. [2] was used. Mice fasted for 18 hours prior to experimentation were divided into five groups of five animals each and treated orally with different doses of RMR, 50 minutes before oral administration of a hydrochloric acid (0.3 M) – ethanol (60%) mixture (0.2 mL/mouse), as follows:

- Groups 1 and 2: received distilled water at 5 mL/kg (negative control; positive control, respectively);
- Group 3: received sucralfate (reference drug, 100 mg/kg);
- Groups 4 and 5: received RMR at 0.23 mL/kg and 1.15 mL/kg, respectively.

Sixty minutes after administration of the ulcerogenic agent (except for the negative control group), the mice were euthanized by cervical dislocation. The stomach of each mouse was excised, opened along the greater curvature with scissors, rinsed with physiological solution, and spread on a white sheet for ulcer observation with the naked eye and under a binocular magnifier (Leica, 2000). Ulcerative lesions appeared in the gastric mucosa as parallel black and red lines along the gastric axis.

2.3.2. Effect of RMR on Ulcers Induced by 50% Ethanol in the Presence of Diclofenac (35 mg/kg) in Rats

Twenty-five rats fasted for 18 hours were divided into five groups of five animals each and treated orally with different doses, as follows: distilled water (negative control, 5 mL/kg), distilled water (positive control, 5 mL/kg), misoprostol (reference drug, 0.5 mg/kg), and RMR at 0.23 and 1.15 mL/kg, 45 minutes before diclofenac treatment (35 mg/kg orally), except for the negative control group. The treatment was administered daily for four consecutive days. On the fifth day, each rat received intragastric administration of 1 mL of 50% ethanol. One hour later, the rats were euthanized by cervical dislocation, their stomachs excised and opened along the greater curvature. Gastric mucus was scraped with a spatula and placed in pre-weighed tubes [2]. The length of each lesion was estimated according to Germano:

0 → no lesion; **4** → more than three small lesions (1);

1 → 1–3 small lesions ≤ 10 mm; **5** → more than three large lesions (2);

2 → 1–3 large lesions ≥ 10 mm; **6** → more than three thick lesions (3);

3 → 1–3 thick lesions (1).

The lesion index (mm) for each stomach was expressed as the sum of the lengths of all lesions. The excised stomachs were also subjected to histopathological examination.

2.4. Microbiological Analysis of the RMR

Microbial detection and enumeration were carried out according to standardized procedures [3].

2.4.1. Enumeration of Microorganisms

Microorganism counts were performed using the decimal dilution or serial dilution technique, consisting of diluting the sample tenfold and inoculating the inoculum of each dilution onto a specific medium. After incubation, microorganisms present in the sample multiplied into

visible colonies that could be counted. Results were expressed in colony-forming units (CFU) per mL of sample [4].

2.4.2. Preparation of Inocula

Inocula were prepared by the serial dilution technique. A volume of 10 mL of the recipe was aseptically collected near a Bunsen burner using a micropipette, then diluted in 90 mL of previously sterilized physiological water. The mixture was homogenized manually for 5 minutes, constituting the mother suspension. From this, serial decimal dilutions were prepared. One milliliter of the mother suspension was transferred into the second tube containing 9 mL of sterile distilled water. The operation was repeated up to 10^{-5}. These dilutions constituted the inocula [4].

The remainder of the mother suspension was pre-enriched by incubating at 37°C for 24 h to detect specific organisms such as Salmonella and Shigella on SS agar. After 24 h of incubation, the pre-enrichment was further enriched as follows: one milliliter of the pre-enrichment was diluted in 10 mL of broth; 0.1 mL of the pre-enrichment was inoculated into 10 mL of broth. The enrichment was incubated at 37°C for 24 h.

2.4.3. Inoculation of Culture Media

Agar plate cultures were prepared using surface inoculation. For this, 0.1 mL of inoculum from a dilution was deposited on the agar surface with a Pasteur pipette. The inoculum was then spread across the agar surface in tight streaks. Only odd dilutions (10^{-1}, 10^{-3}, 10^{-5}) were plated.

2.4.4. Reference of the Microbiological Method

The microbiological analyses of RMR were performed following standardized AFNOR [3] procedures, specifically NF.V08 recommendations, under defined incubation conditions. For total aerobic mesophilic flora, incubation was carried out at 37°C for 24 h, allowing enumeration of bacteria forming white colonies. Total coliforms were incubated at 37°C for 24 h, while fecal coliforms (thermotolerant) required incubation at 44°C for 24 h, a condition favoring the growth of enterobacteria indicative of fecal contamination, showing violet to red-pink colonies. For Staphylococcus aureus, selective media were incubated at 37°C for 24 h, where pathogenic colonies appeared pigmented yellow. The detection of Salmonella and Shigella involved enrichment followed by incubation at 37°C for 24 h on selective media, allowing their isolation. Finally, yeast and mold enumeration was performed at 37°C for 72–120 h, with identification based on the presence of white colonies.

2.4.5. Observation, Colony Counting, and Estimation of Microorganisms

Colonies on each Petri dish were observed visually and counted for the same dilution. One dish was considered per dilution. The number of microorganisms was calculated from colony counts on plates corresponding to dilutions yielding significant results. Each colony was considered to originate from one or more microorganisms, thus forming a CFU. According to AFNOR [3], only plates with more than 30 and fewer than 300 colonies were retained. The number of microorganisms in a given product sample was calculated using the formula [5]:

N (ufc / mL) = Σ des colonies / V (n1+ 0,1 n2).d

Where:

N: number of microorganisms;

V: volume of dilution used (0.1 mL surface plating, 1 mL in-depth inoculation);

n1: number of plates in the first dilution;

n2: number of plates in the second dilution;

d: dilution factor corresponding to the first dilution retained.

2.5. Microbiological Standards

The number of microorganisms per mL or g was calculated for each flora studied, according to the analyzed samples from each locality, and compared to the normative reference of microbiological criteria for plant-based medicines of the European Pharmacopoeia [6].

2.6. Evaluation of Physicochemical Quality

The physicochemical quality analysis was performed on the recipe.

2.6.1. Hydrogen Potential (pH)

The pH was measured on 3 mL of filtrate placed in a 10 mL beaker using a pH meter equipped with a combined electrode (HI83141 type).

2.6.2. Conductivity

Conductivity, which indicates the content of soluble salts in the product, was measured on 3 mL of filtrate placed in a 10 mL beaker using a conductimeter. Results were expressed in mS/cm.

2.6.3. Solvent Content

The analysis of solvent content consisted of evaporating the solvent from the recipe and weighing the remaining non-volatile residues to determine solvent purity and assess contaminants. Solvent content was calculated using the following formula:

$$(T) = \frac{M0 - M_1}{M_1}$$

Where:

T: solvent content;

M_0: initial mass of the sample;

M_1: mass of the dried sample.

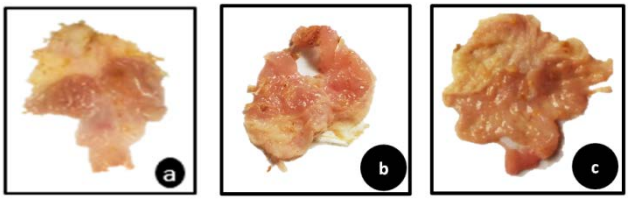
2.7. Phytochemical Screening

The main chemical groups present in the recipe were identified by coloration and precipitation reactions in test tubes using specific chemical reagents, according to the methods reported by Bassene [7] and Hamid and al. [8].

3. Results

1. Effect of RMR on Gastric Mucosa in the Acute Model in Mice

Macroscopic examination of the gastric mucosa revealed no lesions in mice that received distilled water (Figure 1a), nor in those treated orally with the medicinal recipe at both doses (0.23 mL/kg and 1.15 mL/kg).

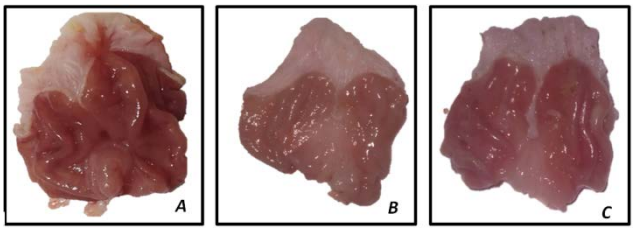


a: Distilled water; **b**: RMR at a dose of 0.23 mL/kg; **c**: RMR at a dose of 1.15 mL/kg

Figure 1. Macroscopic observation of the gastric mucosa in normal mice

2. Effect of RMR on Gastric Mucosa in the Subacute Model in Rats

Macroscopic analysis of the mucosa showed no gastric lesions and a normal appearance in both the control animals (distilled water only; Figure 2A) and those treated with the recipe at the two doses (0.23 and 1.15 mL/kg) (Figure 2B and Figure 2C).

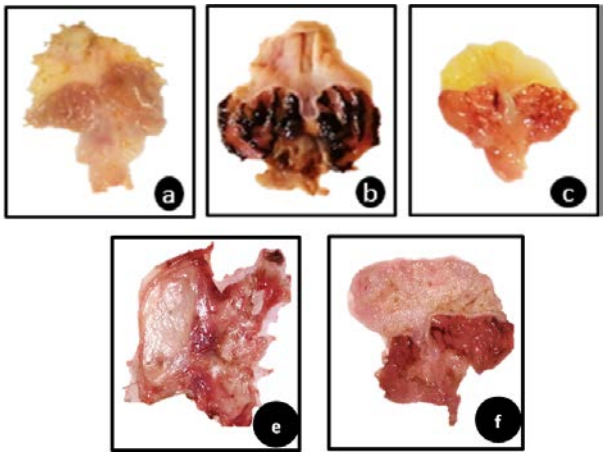


A: Distilled water; **B**: RMR at a dose of 0.23 mL/kg; **C**: RMR at a dose of 1.15 mL/kg

Figure 2. Macroscopic observation of the gastric mucosa in normal rats

3. Effect of RMR on Ulcers Induced by HCl 0.3 M / 60% Ethanol in Mice

Gastric mucosa of mice administered the HCl/ethanol mixture by gavage showed severe lesions (b). Mild ulceration was observed in mice treated with HCl/ethanol combined with sucralfate or RMR at 1.15 mL/kg (c and g), whereas no ulceration was detected in those treated with RMR at 0.23 mL/kg (e).



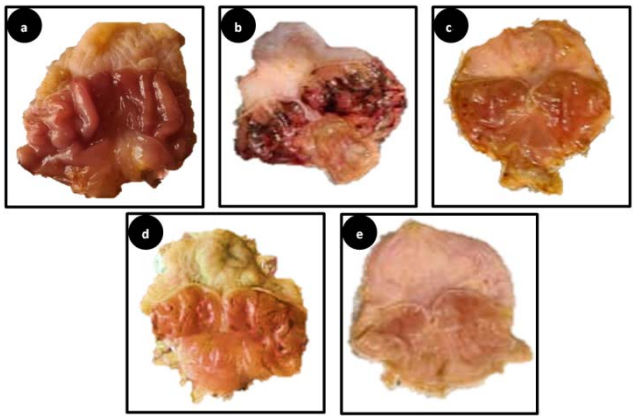
a: distilled water; b: distilled water + HCl/ethanol; c: sucralfate + HCl/ethanol; d and e: RMR (0.23 and 1.15 mL/kg) + HCl/ethanol.

Figure 3. Macroscopic observation of acute gastric mucosal lesions induced by the HCl 0.3 M/ 60% ethanol mixture in mice

3.1. Effect of RMR on Ulcers Induced by Diclofenac/HCl 0.3 in Rats

3.1.1. Macroscopic Analysis of Gastric Mucosa

Oral administration of HCl/ethanol and diclofenac caused lesions and marked necrosis in the stomachs of rats treated with distilled water (b), unlike healthy rats without lesions (a). Conversely, rats pretreated with misoprostol or RMR (0.23 and 1.15 mL/kg) showed reduced lesions (c, d, and e) compared to those exposed only to the injurious substances (b).



a: distilled water; b: distilled water + diclofenac/HCl; c: misoprostol + diclofenac/HCl; d and e: RMR (0.23 and 1.15 mL/kg) + diclofenac/HCl

Figure 4. Macroscopic observation of acute gastric mucosal lesions induced by diclofenac/HCl 0.3 in rats

3.2. Effect of the Revealed Medicinal Recipe on Gastric Lesions in Rats

The results indicate that both misoprostol and RMR, at the tested doses, significantly reduced the ulcer index induced by ethanol and diclofenac. Moreover, they markedly increased gastric mucus production, with respective rates of 75.35%, 81.08%, and 80.67% for misoprostol and RMR at the two doses.

Table 1. Antiulcer effect of the revealed medicinal recipe on ulcers induced by ethanol in the presence of diclofenac in rats

Treatment	Doses (mL; mg/kg; mL/kg)	Ulcer Index (mm)	Mucus Mass (mg)	Mucus Production (%)
DW	5	0.00 ± 0.00	0.19 ± 0.00	/
DW + diclo/EtOH	5	10.68 ± 0.52 ###	0.14 ± 0.00 ###	/
Misoprostol+diclo/EtOH	100	6.55 ± 0.10 ***	0.18 ± 0.00 ***	75.35
RMR + diclo/HCl	0.23	7.67 ± 0.01 ***	0.17 ± 0.00 **	81.08
	1.15	8.55 ± 0.03 **	0.17 ± 0.00 ***	80.67

Values are expressed as mean ulcer index ± SEM (UI ± SEM) ; ###P < 0.001, significant difference compared to the negative control group (DW); ** P < 0.01, *** P < 0.001, significant difference compared to the positive control group (DW + diclo/HCl).DW: distilled water; diclo: diclofenac; EtOH: ethanol; miso: misoprostol; RMR: revealed medicinal recipe.

3.3. Microbiological Evaluation of RMR

The results of microbiological analysis of microorganisms in RMR, expressed in colony-forming units per milliliter (CFU/mL), are presented in Table 2 The findings, also illustrated in the figure, show that total aerobic mesophilic flora (TAMF) (Figure 1A) and yeasts and molds (Figure 1B) were present in the tested samples at levels of 2.2×10^6 CFU/mL and 10^4 CFU/mL, respectively. However, fecal and total coliforms, as well as pathogenic bacteria such as *S. aureus* and *Salmonella*, were entirely absent.

Table 2. Results of microbiological analyses

Parameters	Bacterial Concentration (CFU/mL)	Standards
Total aerobic mesophilic flora (TAMF)	2.2×10^6 CFU/mL	10^3 CFU/mL
Yeasts and molds	10^4	$\leq 10^2$ CFU/mL
Fecal coliforms	0	10^2 CFU/mL
Total coliforms	0	10^2 CFU/mL
Salmonella	Absent	Absent
Staphylococcus aureus	0	10^3 CFU/mL

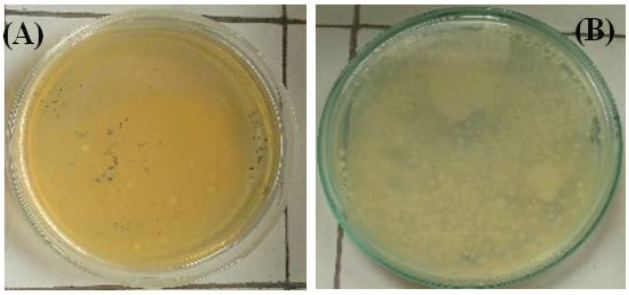


Figure 3. Microbiological quality of different culture media for target microorganisms: (A) BHIA medium: yeast and mold; (B) PCA: TAMF.

3.4. Physicochemical Quality of RMR

The results of physicochemical analyses are summarized in Table 3 The recipe exhibited an acidic pH of 4.61, electrical conductivity of 3625 ms/cm, total dissolved substances of 1835 ppm, and solvent content of 20.04%.

Table 3. Physicochemical analysis results of the recipe

Parameters	Concentration	Standard
pH	4.61	5.5 – 8.5
Electrical conductivity	3.625	$\leq$ 5.2 (ms/cm)
Total dissolved substances	1835	0 – 500 (ppm)
Solvent content	20.04	(%)

3.5. Phytochemical Profile of RMR

Qualitative characterization tests based on precipitation or coloration reactions using specific reagents yielded the results summarized in Table 4 The analysis revealed the presence of several secondary metabolites in the revealed medicinal recipe, including tannins, alkaloids, anthraquinones, oses and holosides, mucilages, and reducing sugars. However, saponosides were absent.

Table 4. Phytochemical profile of RMR

Chemical Compounds	Observation	Results
Tannins	Bluish-black	+
Alkaloids	Yellowish precipitate	+
Flavonoids	Orange coloration	+
Anthraquinones	Red coloration	+
Oses-holosides	Red coloration	+
Mucilages	Flocculent precipitate	+
Reducing sugars	Brick-red precipitate	+
Saponosides	Foam of 1–9 cm	–

+ : Present; – : Absent

4. Discussion

In order to promote the use of medicinal recipes, the anti-ulcer activity, as well as the microbiological, physicochemical, and phytochemical qualities of the revealed medicinal recipe (RMR), were evaluated.

The medicinal recipe did not induce ulcers in the treated animals, as evidenced by macroscopic examination of the gastric mucosa, which revealed no lesions in animals receiving either distilled water (Figure 166a) or RMR at doses of 0.23 mL/kg and 1.15 mL/kg. However, in vivo tests revealed minor ulceration at the 1.15 mL/kg dose, absent at 0.23 mL/kg, suggesting a gastroprotective potential comparable to that of sucralfate. The observed effect may result from the inhibition of inflammatory mediators responsible for gastric lesions [9,10,11]. Moreover, in the combined ethanol and diclofenac model, RMR significantly reduced the ulcer index and stimulated mucus production, an effect similar to misoprostol, corroborating the observations of Alencar and *al*., Ali Khan and *al*. and Mallem [12,13,14].

From a microbiological standpoint, total aerobic mesophilic flora and yeasts/molds slightly exceeded AFNOR [3] standards, indicating potential contamination related to preparation conditions, storage, or raw materials [15,16]. Conversely, the absence of coliforms, Salmonella, and pathogenic Staphylococcus indicates adherence to hygiene practices during handling [17,18]. The acidity of the RMR may also explain this absence, as acidic pH is recognized for its antiseptic properties [19].

Physicochemical analysis confirmed an acidic pH, slightly below European Pharmacopoeia standards [6]. This acidity, likely related to the nature of the ingredients (e.g., substances rich in organic acids), may also be influenced by production hygiene and microbial activity [20,21]. Electrical conductivity (3.625 µS/cm) falls within the standard range, but the dissolved solids content exceeds requirements, which could be due to the addition of solvents such as hydrocarbons, affecting solubility and product stability.

Finally, phytochemical analysis revealed the presence of tannins, flavonoids, alkaloids, anthraquinones, mucilages, and reducing sugars, with an absence of saponosides. Tannins and mucilages, already known for their gastroprotective properties [22,23,24], may contribute to the observed effects.

5. Conclusion

At the end of this study, the results demonstrated that the revealed medicinal recipe (RMR) possesses gastroprotective properties. The product's quality was found to be moderately satisfactory in terms of microbiological parameters, although it varied depending on the microorganisms analyzed. Physicochemical analyses indicated that the RMR is characterized by an acidic pH, electrical conductivity within the standard range, a total dissolved solids content considerably above normal, and a solvent content of 20%. Phytochemical investigation revealed the presence of multiple secondary metabolites, which could account for the observed pharmacological effects.

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