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Research Article

Evaluation of toxicity, antispasmodic, and analgesic activities of Improved Traditional Medicine (ITM) based on *Diospyros mespiliformis* and *Combretum micranthum* indicated in the treatment of gastrointestinal disorders

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Abstract



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This study focused on an improved traditional herbal medicine (ITHM) used in capsule form for the treatment of gastrointestinal disorders. This study aimed to investigate the phytochemistry, toxicity, and pharmacological activity of the ITM. ITM and extracts underwent phytochemical characterization tests and their dosages. Antioxidant, antispasmodic and analgesic properties and acute toxicity study were realized. Bioactive compounds, including flavonoids, saponins, tannins, etc. were detected. The total phenolic of the ITM were higher with 109.02 ± 0.15 mg EAT/g. The highest total flavonoid content was obtained by the decocted with 17.04 ± 0.25 mg EQ/g and ITM with 16.91 ± 0.80 mg EQ/g. ITM had the best antioxidant activity by the DPPH method with an IC_{50} of 24.65 ± 1.05 μ g/ml; the decocted had the best activity by the ABTS method with an IC_{50} of 12.86 ± 0.95 μ g/ml and the extracts and ITM had significantly the same activities by LPO method, hence the choice of ITM for the rest of the pharmacological activity. ITM has a low toxicity and an estimated LD_{50} of 5000 mg/kg. The maximum antispasmodic effect of ITM at the maximum concentration of 3.98 mg/mL was 87.30% with an effective concentration 50 (EC_{50}) of 0.66 ± 0.05 mg/mL. The analgesic effect of ITM was dose-dependent, with inhibition percentages at the dose of 50 mg/kg of 59.82%, that of 100 mg/kg of 64.73% and that of 150 mg/kg of 71.43%. The results on ITM provide a scientific basis for its use in the management of gastric pathologies.

Keywords: ITM; Phytochemicals; Antioxidants; Acute toxicity; Antispasmodic; Analgesic.

INTRODUCTION

Gastrointestinal (GI) diseases have a range of disorders that affect the digestive tract, from the esophagus to the anus ¹, which constitutes a public health problem. Various factors, including infections, inflammatory disorders, motility problems, structural abnormalities, and lifestyle factors, can cause these to range in severity from mild to severe ². These pathologies have the

potential to lead to serious complications, such as gastrointestinal bleeding, intestinal obstructions, perforations, and even cancers, especially in the colon ³. Chronic pain, symptoms, and illness worries can negatively impact mental health, resulting in stress, anxiety, and depression ⁴. These conditions can cause a significant increase in healthcare expenses due to

medical consultations, diagnostic tests, treatments, and sometimes hospitalizations ⁵.

It is crucial to take measures to guarantee equitable and affordable access to essential treatments in a global context that is plagued by the scarcity of financial resources and difficulties in accessing medicines ⁵. To achieve this, it involves strengthening health systems, improving medicine management, and promoting policies that promote innovation and local medicine production. In this context, natural compounds from medicinal plants are a promising alternative ⁵. The use of plant resources as a first choice by the populace, particularly in developing countries, is based on historical evidence of effectiveness, acceptability, accessibility, and cultural compatibility, based entirely or partially on phytotherapy ^{6, 7}. The main therapeutic resources for treating and managing various diseases are medicinal plant products. Traditional medicine practitioners (TMPs) use these plants both individually and in combination. The rationale for this is that synergies of action can lead to an increase in the therapeutic effect or a reduction in the side effects caused by certain plants ⁸. The pharmacological activity of a single plant may be greater or lesser than that of a combination of other plants. A better therapeutic effect can be obtained through synergy and/or toxicity can be reduced by using this combination of several plants in a precise dosage ^{9, 10}.

Although liquid products derived from medicinal plants are one of the main ways to use them in traditional use ⁸, the powder form is the most common and provides better conservation.

Plant powder (alone or combined) is frequently utilized directly by PMTs for the treatment of multiple pathologies, such as abdominal pain and diseases with inflammatory components, through direct chewing or rapid dispersion with water or other liquids ¹¹. An IMT made of powder from *Diospyros mespiliformis* and *Combretum micranthum* has been developed by a PMT for treating gastrointestinal pathologies. This MTA has demonstrated a long duration of use in traditional environments. The two plants taken separately are used for their antispasmodic, anti-inflammatory properties, etc. ^{12, 13}. In the process of promoting traditional medicine, it would be appropriate to evaluate the safety and effectiveness of ITM through scientific evidence. This will allow for an improvement in quality through control of all parameters and properties, which would guarantee the safety of populations and bring traditional medicines to a satisfactory level of requirement ¹⁴.

This study is aimed at evaluating ITM's phytochemical properties, safety (acute toxicity), and efficacy (antioxidant, antispasmodic, and analgesic).

METHODOLOGY

Plant material

ITM comes in powder form, containing a mixture of *Diospyros mespiliformis* Hochst ex A. DC (Ebenaceae) and *Combretum micranthum* G. Don. (Combretaceae). It was provided by the phytotherapy and alternative care

laboratory "LAPHYSOA" of the traditional health practitioner.

Phytochemical characterization test

Macroscopic and organoleptic analyses of ITM

The macroscopic and organoleptic characteristics (color, odor, texture, and flavor) were determined.

Determination of the residual moisture content (RMC) of the ITM

The residual moisture content was determined using the method based on water loss on drying. A one-gram (1g) test portion of the powder was weighed in triplicate into an aluminum cup and placed in the halogen desiccator at 105°C for 15 min. The RMC was read after the beep.

Extraction

Decoction: A test sample of 25 g of ITM was dispersed in 250 ml of distilled water. The whole was brought to a boil for 30 minutes. After cooling, the decoction was filtered, and the filtrate was collected and then centrifuged at 2000 rpm for 10 minutes. The centrifuged filtrate was dried in a ventilated oven to obtain the dry extract, and the extraction yield was calculated.

Maceration: A 25 g test sample of ITM was dissolved in 250 ml of distilled water; the mixture was left to macerate for 24 h at room temperature. After 24 h, the mixture was filtered and centrifuged at 2000 rpm for 10 min. The obtained filtrate was placed in the oven at 50°C to obtain the dry extract, and the yield was calculated.

Phytochemical test

Phytochemical screening

Phytochemical screening of ITM and the extracts was carried out on chromatoplates (60 F250, 20x20 glass support, Fluka-Silica gel) according to the methods described in the literature ¹⁵. This involved searching for large chemical groups by thin layer chromatography (TLC), such as steroid compounds, terpene compounds, saponosides, and phenolic compounds. The solvent system consisting of Ethyl Acetate-Methanol-Water (7-2-1) was used for the migration of the compounds.

Several specific reagents have been used to reveal these groups of compounds: Sulfuric vanillin reagent and Libermann Burchard reagent for terpenes and sterols; methanolic 5% (V / V) KOH reagent for coumarins; Neu reagent for flavonoids; FeCl₃ reagent for tannins and phenolic compounds, and sulfuric anisaldehyde reagent for saponosides.

Dosage of phytochemical compounds

Total phenolics

Total phenolics were determined according to the method of Singleton *et al.* ¹⁶. The reaction mixture consisted of 1 ml of ITM or each extract, 1 ml of 2N FCR, and 3 ml of 20% sodium carbonate solution. It was left to stand at room temperature for 40 min, and then the absorbance was measured at 760 nm using a spectrophotometer (Agilent 8453). In the white control tube, the extract was replaced by distilled water. A

standard curve was plotted with tannic acid (1.5 µg/ml). The tests were carried out in triplicate. The concentration of total phenolics in the extract was determined.

Total flavonoids

The flavonoid dosage was carried out according to the method of Kumaran *et al.*¹⁷ adapted by Abdel-Hameed¹⁸. Two (2) ml of ITM or each extract of concentration 1 mg/ml in methanol were mixed with 2 ml of 2% aluminum trichloride in methanol. After 40 min, the absorbance was measured at 415 nm using the spectrophotometer (Agilent 8453). The blank control tube consisted of 2 ml of methanol. The absorbance of quercetin (0.10 mg/ml) used as a reference compound was measured under the same conditions. The tests were carried out in triplicate. The amount of flavonoids in the plant extract in Quercetin Equivalent (EQ) was determined.

Antioxidant test

Reduction of the DPPH radical (2,2-diphenyl-1-picrylhydrazyl)

The method of Kim *et al.* was used for the determination of the ability of extracts to reduce DPPH free radicals¹⁹. A dilution series was performed from the ITM or each extract and Trolox. Twenty (20) µL of these solutions (extracts and Trolox) were placed in wells of a 96-well microplate, all supplemented with 200 µL of the DPPH solution (0.04 mg/ml). After 30 minutes of incubation, the absorbance was read using a Biorad 680 spectrophotometer at 490 nm. The blank was prepared with 200 µL of DPPH and 20 µL of 99.9% pure methanol. A curve of percentage inhibition of DPPH was then plotted as a function of sample concentration. The latter made it possible to determine the concentration necessary to degrade 50% DPPH (IC₅₀).

Reduction of the ABTS radical (2,2'-azinobis- [3-ethylbenzothiazoline-6-sulfonic Acid])

The method used is that described by Re *et al.* and Arts *et al.*^{20, 21}. Different dilution series were prepared from a stock concentration of ITM or each extract or Trolox at 1 mg/ml. A volume of 200 µL of ABTS solution was added to 20 µL of the recipe or each extract at different concentrations or with 20 µL of trolox in 96-well plates. The whole was then incubated for 30 minutes at 25°C, and the absorbances were read using the Biorad 680 spectrophotometer at 415 nm. The blank control consisted of a mixture of 20 µL of 96° ethanol and 200 µL ABTS. All measurements were carried out in triplicate. The absorbance inhibition curve versus the concentration of extracts or Trolox was plotted to determine the 50% inhibitory concentration (IC₅₀).

Inhibition of lipid peroxidation (LPO)

Lipid peroxidation inhibitory activity of rat liver was determined using 2-thiobarbituric acid. FeCl₂-H₂O₂ was used to induce peroxidation of liver homogenate according to a method adapted by Sombié *et al.*²² with some modifications. An amount of 0.2 ml of ITM or each extract at the concentration of 1.5 mg/ml was mixed with

1 ml of 1% Wistar rat liver homogenate, then 50 µL of FeCl₂ (0.5 mM) and 50 µL of H₂O₂ (0.5 mM) were added thereto. The mixture was incubated at 37°C for 60 min, then 1 ml of trichloroacetic acid (15%) and 1 ml of 2-thiobarbituric acid (0.67%) were added. The reaction mixture was heated in boiling water for 15 min. Absorbance is read at 532 nm with a BioRad 680 Spectrophotometer. Trolox was used as a reference product. The ability of the extracts to inhibit liver lipid peroxidation was determined as a percentage inhibition.

Acute oral toxicity test

The acute toxicity test of ITM was performed on female NMRI mice according to OECD Guideline 423²³. After 4 h of fasting, the ITM extract was administered orally by gavage in a single dose to the mice according to the sequential procedure. When carrying out the test, a dose of 2000 mg/kg body weight of extract was chosen as the starting dose. The animals were observed individually during the first 2 hours after administration of the extract for signs of toxicity (changes in skin and coat, eyes, mucous membranes, salivation, convulsions, diarrhea, lethargy, sleep, and coma), and after that, food was restored. They were then observed at least once a day for 14 days to detect mortality.

Evaluation of *in vitro* biological activity

Antispasmodic activity on isolated duodenum

The duodenum was prepared according to the procedure described by Nitiéma *et al.*¹². The rats were fasted for 16 h before the start of the experiment. After cervical dislocation, the duodenum was removed and placed in Tyrode's solution. The isolated duodenum was freed from adherent connective tissue. Rings measuring 1.8 ± 0.2 cm were collected. Each ring was mounted in an isolated organ tank containing 25 mL of Tyrode's solution aerated by a pneumatic pump and maintained at 37 °C.

The base tension was adjusted to 1 g, and Tyrode's solution was changed every 15 min for 45 min of equilibration. The KCl (80 mM) test was performed to verify the ability of the ring to contract appropriately. Induced spontaneous contractions were recorded using an isometric force sensor (Harvard dual-channel oscillograph recorder) connected to an amplifier (Harvard Transducer). The duodenum was treated with 1µM Acetylcholine for maximal contraction. Relaxation curves were recorded by progressive and cumulative addition of ITM (10⁻² to 4 mg/mL) and Spasfon (10⁻³ to 1 mg/mL) over 7 min and additions were made with one (1) minute intervals. The tissue was then washed by changing the bath solution three (3) times and left to stand for 15 min before the next stimulation. The experiment was repeated at least 3 times for each type of extract. The results are expressed as the contraction force as a function of the concentration of the extract and are calculated from the equation below:

$$FC = \frac{\text{Contraction strength of extracts} - \text{Basic contraction}}{\text{duodenum size}}$$

$$FC = \text{contraction force } ([A(\text{cm})/\text{duodenum}(\text{cm})])$$

Evaluation of *in vivo* biological activity

Non-morphine analgesic tests (acetic acid test)

The analgesic effect was evaluated on the number of abdominal contortions induced by intraperitoneal injection of acetic acid (0.6%) following the method described by Ouédraogo *et al.* ²⁴ and modified by Traoré *et al.* ²⁵. NMRI mice were fasted for 16 to 17 hours before the experiment. Groups of six mice were formed. The blank control group received distilled water, and the group receiving the reference substance (paracetamol) at a dose of 100 mg/kg. Different doses of 50, 100, and 150 mg/kg were administered orally to the groups of mice receiving ITM.

One hour after administration of ITM and reference, the animals received acetic acid intraperitoneally at a dose of 10 ml/kg. Five minutes after the injection of acetic acid, the number of writhing movements was counted in each mouse for 15 minutes. The analgesic effect was evaluated according to the following formula:

$$\% \text{ inhibition} = ([W_b - W_t]/W_b) \times 100$$

W_b represents the average number of contortions of mice in the white control group, and W_t is the average number of contortions of mice in the treated group.

Statistical analysis

Experiments were carried out in triplicate and results expressed as mean ± SEM. The analysis of the results was

done on the basis of statistical processing of Graph Prism software version 9, and one-way Analysis of Variance followed by Bonferroni's test was used as a statistical treatment. The differences were considered significant when $p \leq 0.05$ compared to the control.

RESULTS

Macroscopic and organoleptic characteristics

The macroscopic and organoleptic characteristics of ITM are presented in Table 1.

Table 1: macroscopic and organoleptic characteristics of the recipe

ITM Parameters	Organoleptic Characteristics
Odor	Odorless
Texture	Smooth and fine
Appearance	Uniform to the naked eye
Taste	No particular taste
Color	Brown

The ITM was brown with a rather uncharacteristic odor and no particular taste. It was smooth, thin, and uniform in appearance to the naked eye.

Physicochemical characteristics

The percentages of moisture content and yield of the samples are recorded in Table 2.

Table 2: Results of residual moisture content and extraction yields

Parameters	RMC in %	Yield in %	
		Decoction	Maceration
Values	7.65 ± 0.74	12.17 ± 0.11	11.12 ± 0.10

The RMC of ITM was 7.65 ± 0.74, and the extraction yields were 12.17 ± 0.11 and 11.12 ± 0.10, respectively, for the decoction and maceration.

Phytochemistry

TLC analysis showed the presence of the phytochemical compounds sought in Table 3

Table 3: Results of chemical groups highlighted by TLC

Compounds	ITM	Decocted extract	Macerated extract
Coumarins	+	+	+
Saponins	+	+	+
Sterols and Triterpenes	+	+	+
Tannins	+	+	+
Flavonoids	+	+	+

Phytochemicals such as saponins, flavonoids, tannins, coumarins, sterols, and triterpenes were highlighted in ITM and both extracts (decoction and maceration).

Contents of phytochemical compounds of interest

The results of the determination of phenolic compounds are recorded in Table 4.

Table 4: Total phenolic and total flavonoid content

	ITM	Decoction extract	Macerated extract
Total phenolics (mg EAT/g)	109.02±0.15	105.59± 1.87	93.01± 0.49
Total flavonoids (mg EQ/g)	16.91±0.80	17.04±0.25	14.22±0.94*

The highest content of total phenolics was obtained by ITM with 109.02± 0.145 mg EAT/g and the lowest content by the macerated extract with 93.01± 0.49 mg EAT/g. The flavonoid content ranged from 14.22±0.94 with the macerated extract to 17.04±0.25 mg EQ/g with the decoction. Statistical analysis showed no difference between the flavonoid content of ITM and that of the

decoction extract. Both ITM and decoction extract have higher content of total phenolics and total flavonoids.

Antioxidant activities

The results of the antioxidant activity of ITM and different extracts evaluated by the ABTS, DPPH, and LPO methods are reported in Table 5.

Table 5: Results of antioxidant activities of powders

	DPPH	ABTS	LPO
	IC ₅₀ µg/ml	IC ₅₀ µg/ml	%
ITM	24.65 ± 1.05	32.39 ± 0.93	53.16 ± 0.15
Decoction extract	28.71 ± 1.02	12.86 ± 0.95	55.12 ± 1.01
Macerated extract	29.62 ± 0.97	42.23 ± 0.99	53.31 ± 0.30
Trolox	3.3 ± 0.05	2.33 ± 0.25	66.26 ± 3.01

For the antiradical activity by inhibition of the DPPH radical, ITM had a higher activity with an IC₅₀ of 24.65 µg/ml, and the lowest activity was obtained with the macerated extract with an IC₅₀ of 29.62 µg/ml. There is no statistically significant difference between the activity of the decoction (28.71 ± 1.02) and macerated (29.62 ± 0.97) extracts. The reference compound (trolox) has a higher activity than ITM and the studied extracts (3.3 µg/ml).

With the ABTS radical inhibition method, the results show that the decoction has a better antioxidant activity (12.86 ± 0.95 µg/ml), followed by ITM (32.39 ± 0.93 µg/ml) and the lowest activity with the macerated extract (42.23 ± 0.99 µg/ml).

For the lipid peroxidation inhibition test, ITM, macerate, and decoction have an inhibitory activity greater than 50% but trolox has a higher activity, i.e., a percentage of 66.26%. There is no statistical difference between the activity of ITM and that of the two extracts.

Acute oral toxicity

The dose of 2000 mg/kg body weight (bw) did not cause any mortality or significant behavioral changes in female mice during the first and second administration of ITM.

Figure 1 shows the average weight observed over 14 days in control female mice and those receiving ITM at a single dose of 2000 mg/kg. No statistically significant difference

was observed between the treated and control groups in terms of body weight gain. ITM administration did not influence the food and water consumption of mice.

Macroscopic examination of vital organs such as heart, lungs, liver, kidneys, and spleen of control mice and mice treated with ITM at 2000 mg/kg did not show any lesions, nor any change in color or appearance of the different organs. Table 6 summarizes the relative organ weights of control and test mouse groups. No statistically significant variation was observed between the relative organ weights of the control and treated groups.

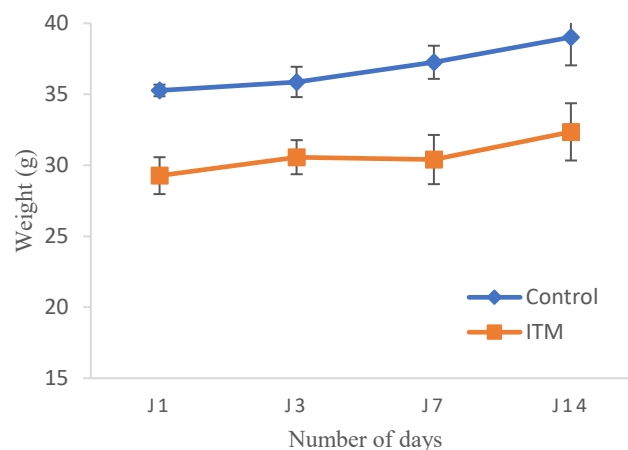


Figure 1: Average weight observed over 14 days of the mice tested

Table 6: Relative weight of organs of the mice tested

Organs	Weight (mg)	
	Control	ITM
Heart	0.38±0.05	0.37±0.06
Lungs	0.62±0.08	0.59±0.09
Liver	4.86±0.68	4.60±0.23
Spleen	0.44±0.03	0.47±0.10
Kidneys	1.04±0.18	0.92±0.08

Antispasmodic activity

The results of relaxation of isolated rat duodenum using ITM and Spasfon are shown in Figure 2 along with those of 50% effective concentrations.

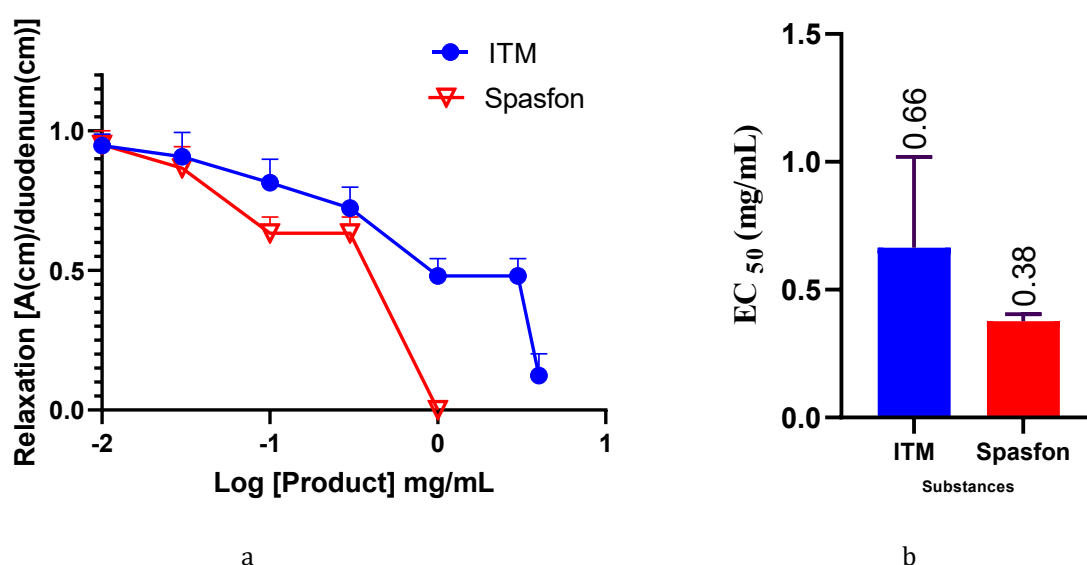


Figure 2: Relaxation curve (a) of the duodenum and the effective concentration 50 (b) of relaxation

Figure 2a shows the relaxation curves of ITM and the reference compound (Spasfon used as a control) on the isolated rat duodenum pre-contracted with acetylcholine, while Figure 2b, represented as a histogram, gives the effective concentration 50 (EC₅₀) of both. The EC₅₀ of ITM was 0.66±0.05 mg/mL, and that of Spasfon was 0.38±0.01 mg/mL.

The maximum effect of ITM at the maximum concentration of 3.98 mg/mL was 87.30% while that of

the reference at the maximum concentration of 1 mg/mL was 100%. A statistically significant difference was noted between the effect of ITM and that of Spasfon.

Analgesic activity

Figure 3 and Table 7 illustrate the non-morphine analgesic effect of ITM and the reference (paracetamol) after injection of acetic acid.

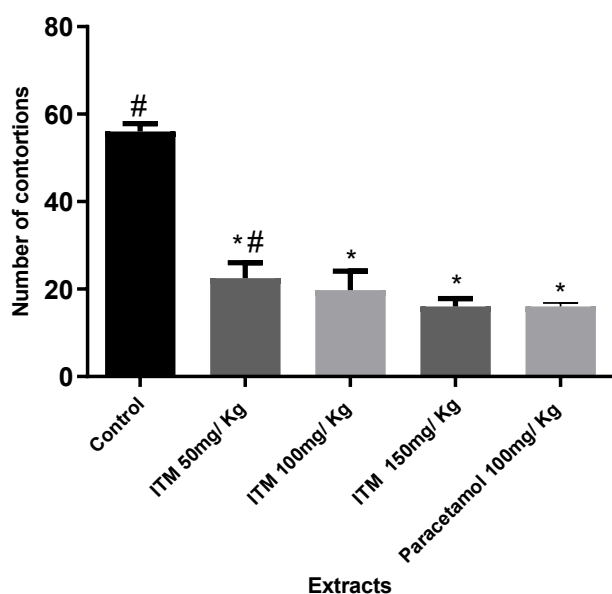


Figure 3: Number of contortions

*Values are mean $\pm$ S.E.M. $n = 6$. *: $p < 0.05$ indicates a significant difference from normal control (one-way ANOVA analysis followed by Bonferroni test) and # indicates a significant difference from paracetamol

The writhing number due to pain (Figure 3) was 22.5 ± 3 , 19.75 ± 3.13 , and 16 ± 0.15 , respectively, for ITM at 50, 100, and 150 mg/kg doses compared to the reference compound, which gave a writhing number of 16 ± 0.5 at a 100 mg/kg dose.

Pain inhibition percentages were all greater than 50% with the doses of ITM used (Table 7). The percentage of inhibition of ITM at the dose of 50 mg/kg was 59.82%, that of 100 mg/kg was 64.73% and that of 150 mg/kg with a percentage of inhibition 71.43% equal to that of paracetamol at the dose of 100 mg/kg, which was 71.43%.

DISCUSSION

The identification and differentiation of herbal drugs can be determined by analyzing organoleptic and macroscopic characteristics, which can also determine their degree of purity by analyzing the presence/absence of foreign elements, as well as by detecting adulteration or falsification²⁶. ITM was a powder that was smooth and fine, with a uniform appearance, a brown hue, an uncharacteristic smell, and no specific taste. The presence of these characteristics would indicate the preparation of ITM to be homogeneous and uniform, which would be beneficial for its acceptability and ease of use¹⁴. These characteristics may serve as a fingerprint for ITM, enabling it to be distinguished from other preparations.

The ITM's RMC of less than 10% will be able to indicate better stability against possible degradation and good preservation of the latter. The ITM RMC complied with the standards established by the International Pharmacopoeia 10th Edition²⁷.

The determination of yields allows the quantitative assessment of extractable compounds from ITM²⁸. The

Table 7: % of contortion inhibition

	Dose mg/kg	% inhibition
ITM	50	59.82
	100	64.73
	150	71.43
Paracetamol	100	71.43

ITM extraction yields were 12.17 ± 0.11 for the decoction and 11.12 ± 0.10 for the maceration. The decoction that yielded the best would be the appropriate method using heat to extract the most extractable compounds²⁹. These yields constitute an indicator of the effects of extraction conditions and would allow a more rational use of medicinal plants³⁰.

Phytochemicals such as saponins, flavonoids, tannins, coumarins, sterols, and triterpenes were highlighted in ITM and both extracts (decoction and maceration). The decoction extraction method, from a qualitative point of view, is as effective as maceration³¹. The same secondary metabolites were highlighted, and molecules were isolated in the extracts of the two plants constituting ITM³²⁻³⁴. Despite the extraction process, the compounds present in the extracts were also present in the ITM, which would explain why the extraction did not denature the phytochemical compounds present in the latter. The presence of these phytochemicals would justify the use of ITM in the management of abdominal pain. Indeed, the chemical groups highlighted are known for their antispasmodic properties³⁵. Also, phenolic compounds play a major role in protection against certain diseases due to their possible interaction with numerous enzymes and their antioxidant properties³⁶, analgesic³², etc., hence their choice as compounds of interest.

The highest content of total phenolics was obtained by ITM with 109.02 ± 0.145 mg EAT/g and that of flavonoids by the decoction with 17.04 ± 0.25 mg EQ/g. Statistical analysis showed no difference between the flavonoid content of ITM (16.91 ± 0.80 mg EQ/g) and that of the decocted extract. Both ITM and decoction extract have higher total phenolic and total flavonoid content. Phenolic compounds, depending on their structures,

could have a high degree of free radical scavenging activity. ITM could be chosen for biological activity.

ITM and decoction had the highest anti-radical activities by the ABTS and DPPH methods, and the 3 extracts (ITM, decoction, and macerated) had the same lipid peroxidation inhibitory capacities. Total phenolics, especially flavonoids, possess hydroxyl groups, which can decompose peroxides, repair oxidative damage, and quench singlet and triplet oxygen³⁷. These antioxidants are promising therapeutic agents, reducing free radicals and modulating inflammatory pathways, spasms, and associated pain³⁸. ITM and decoction would reduce the production of free radicals, which promote the appearance of various associated inflammatory diseases. The unextracted ITM was as rich in phytochemicals as the extracts, had a high content of phenolic compounds, and good antioxidant activity, like the decocted extract. The choice of this ITM in its traditional form could be explained by the in vitro tests carried out.

After administration of a single dose of 2000 mg/kg body weight (bw) to mice, no morphological or behavioral changes or toxicological signs were observed for 14 days. Also, ITM had no impact on body weight, as well as the weight of the different organs of the mice tested. According to OECD Guideline 423, the LD₅₀ was estimated at 5000 mg/kg body weight, and this allows ITM to be classified in category 5 of the OECD and United Nations Globally Harmonized System (GHS)^{23,39}. Previous studies on the acute toxicity of aqueous extracts of the two-component plants of ITM have also shown that these extracts have an LD₅₀ greater than 5000 mg/kg^{12, 40}. These results suggest that ITM may have relatively low acute oral toxicity.

ITM, at the maximum concentration of 3.98 mg/mL, had a relaxant effect of 87.30% on the isolated rat duodenum pre-contracted by acetylcholine with an EC₅₀ of 0.66±0.05 mg/mL. Acetylcholine binds to these muscarinic receptors in the muscle plasma membrane, opening Na⁺ channels, causing membrane depolarization, resulting in the release of Ca²⁺ from the sarcoplasmic reticulum, which results in contraction of muscle cell myofibrils^{35, 41}. ITM causes smooth muscle relaxation. This relaxant effect of ITM may be due to an inhibitory action on the contractile mechanism or to the suppression of contractile stimuli through the reduction of Ca²⁺ release⁴². Moreover, terpenoids, flavonoids, and alkaloids were the chemical groups with the highest number of antispasmodic compounds³⁵, so the antispasmodic activity of ITM could be due to the highlighted phytochemicals. Also, *Disopyros mespiliformis* extracts have myorelaxant effects on isolated rat duodenum pre-contracted with acetylcholine with an efficacy of 79.18% ± 9.33%¹², and the aqueous extract of *Combretum micranthum* induced relaxation of isolated rabbit jejunum and guinea pig ileum but no effect on jejunum pre-contracted with acetylcholine¹³.

Spasms (involuntary muscle contractions) are usually accompanied by pain and interfere with voluntary, free, and efficient muscle activity³⁵. ITM had an analgesic effect on acetic acid-induced pain. The action of ITM resulted in a dose-dependent decrease in the number of

contortions with a percentage of inhibition greater than 50% at the dose of 50 mg/kg bw. ITM at a dose of 150 mg/kg bw has the same percentage of inhibition of writhing (71.43%) as the reference compound, which is paracetamol. ITM is reported to have an inhibitory effect on pro-inflammatory chemical mediators (histamine, serotonin, bradykinin, substance P, and prostaglandins)^{24, 25} that are involved in spasms⁴³. The inhibitory effects of ITM could be attributed to its inhibitory action on enzymes involved in the synthesis of prostaglandins and leukotrienes²⁵. *Disopyros mespiliformis* extracts had in vitro anti-inflammatory activity on pro-inflammatory enzymes (phospholipase A₂ and 15-lipoxygenase)¹², and *Combretum micranthum* extract had an analgesic effect at a dose of 200 mg/kg¹³. The use of ITM, a mixture of the two plants, could be justified by the pharmacological properties highlighted.

CONCLUSION

This research on ITM aimed at treating gastric problems revealed information on its composition, physicochemical properties, and therapeutic potential. The macroscopic examination revealed a homogeneous appearance, with organoleptic characteristics that favor its acceptability to patients. Phytochemical screening confirmed the presence of bioactive compounds such as flavonoids, tannins, sterols, triterpenes, saponosides, and coumarins, suggesting therapeutic potential. Analysis of total phenolic and flavonoid contents revealed non-significant variations between ITM and decocted extract but significant variations between ITM and macerated extract which may indicate differences in therapeutic efficacy with the latter. The results of antioxidant activity highlighted the ability of ITM to act as an effective antioxidant, potentially beneficial for the treatment of gastric diseases, hence the choice of ITM for pharmacological and toxicological activities. ITM was of low toxicity with an estimated LD₅₀ of 5000 mg/kg body weight. It had very significant antispasmodic activity and analgesic activity comparable to that of the reference compound paracetamol. The results on ITM provide a scientific basis for its use in the management of gastric pathologies. Nevertheless, further research on antimicrobial, antidiarrheal, and antidysenteric activity should be considered in order to confirm and fully exploit the therapeutic potential.

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REFERENCES

1. Leader G, Murray M, O'Suilleabháin PS, Maher L, Naughton K, Arndt S, et al. Relationship between parent-reported gastrointestinal symptoms, sleep problems, autism spectrum disorder symptoms, and behavior problems in children and adolescents with 22q11.2 deletion syndrome. *Research in Developmental Disabilities*. 2020;104:103698. <https://doi.org/10.1016/j.ridd.2020.103698> PMID:32474230
2. Mazzocchi S, Visaggi P, Baroni L. Plant-based diets in gastrointestinal diseases: Which evidence? *Best Practice &*

- Research Clinical Gastroenterology. 2023;62-63:101829. <https://doi.org/10.1016/j.bpg.2023.101829> PMID:37094909
3. Peery AF, Crockett SD, Murphy CC, Jensen ET, Kim HP, Egberg MD, et al. Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2021. *Gastroenterology*. 2022;162(2):621-44. <https://doi.org/10.1053/j.gastro.2021.10.017> PMID:34678215 PMCID:PMC10756322
 4. Tiwari BR, Naseeruddin Inamdar M, Orfali R, Alshehri A, Alghamdi A, Almadani ME, et al. Comparative evaluation of the potential anti-spasmodic activity of *Piper longum*, *Piper nigrum*, *Terminalia bellerica*, *Terminalia chebula*, and *Zingiber officinale* in experimental animals. *Saudi pharmaceutical journal : SPJ : the official publication of the Saudi Pharmaceutical Society*. 2023;31(9):101705. <https://doi.org/10.1016/j.jsps.2023.101705> PMID:37576742 PMCID:PMC10413155
 5. Julián-Flores A, Aguilar-Zárate P, Michel MR, Sepúlveda-Torre L, Torres-León C, Aguilar CN, et al. Exploring the Therapeutic Potential of Medicinal Plants in the Context of Gastrointestinal Health: A Review. *Plants*. 2025;14(5):642. <https://doi.org/10.3390/plants14050642> PMID:40094542 PMCID:PMC11901797
 6. Agban A, Gbogbo KA, Hoekou YP, Atchou K, Tchacondo T, Batawila K, et al. Évaluation de l'activité antifongique des extraits de *Cassia alata* L. et de *Piliostigma thonningii* (Schumach.) Milne Redh. (Fabaceae) sur *Candida albicans*. *International Journal of Biological and Chemical Sciences*. 2013;7(3):1041-7. <https://doi.org/10.4314/ijbcs.v7i3.12>
 7. Ogu E, Bibinu DS, Chidebe I, Emeje M, Abdullahi M, Roko G. Therapeutic Potential of Medicinal Plants: Current Situation and Outlook. In: Waisundara VY, editor. *Medicinal Plants - Harnessing the Healing Power of Plants*. Rijeka: IntechOpen; 2024.
 8. Tibiri A, Boria S, Traoré TK, Ouédraogo N, Nikiéma A, Ganaba S, et al. Countrywide Survey of Plants Used for Liver Disease Management by Traditional Healers in Burkina Faso. *Frontiers in Pharmacology*. 2020;11(1750). <https://doi.org/10.3389/fphar.2020.563751> PMID:33597863 PMCID:PMC7883685
 9. Shah SS, Manigauha A, Dubey B. Formulation and Evaluation of Antidiabetic and Antihyperlipidemic Activities of Polyherbal Formulation in Streptozotocin induced diabetic rat. *Pharmaceutical and Biosciences Journal*. 2019;26-30. <https://doi.org/10.20510/ukjpb/7/i1/179298>
 10. Parasuraman S, Thing GS, Dhanaraj SA. Polyherbal formulation: Concept of ayurveda. *Pharmacognosy reviews*. 2014;8(16):73-80. <https://doi.org/10.4103/0973-7847.134229> PMID:25125878 PMCID:PMC4127824
 11. Ouédraogo S, Traore TK, Ishola T, Ouédraogo B, Boly ALG, Silué GNA, et al. Optimization of extraction and lyophilization conditions of *Feretia apodanthera* (Rubiaceae) leaf powders for galenic formulation. *Journal of Drug Delivery and Therapeutics*. 2024;14(2):89-97. <https://doi.org/10.22270/jddt.v14i2.6401>
 12. Nitiéma M, Ouédraogo PE, Traoré TK, Noura OM, Kaboré B, Bélem-Kabré WLME, et al. Phytochemical Profile, Antioxidant, and Anti-Inflammatory Activities, Safety of Use and Spasmolytic Effects of Aqueous Decoction Extract of *Diospyros mespiliformis* Leaves Hochst. ex A. DC. (Ebenaceae) on the Isolated Duodenum of Rat. *Pharmacology & Pharmacy*. 2023;14(12):513-29. <https://doi.org/10.4236/pp.2023.1412034>
 13. Abdullahi M, Anuka J, Yaro A, Musa A. Effect of aqueous leaf extract of *Combretum Micranthum* g. don (Combretaceae) on gastro intestinal smooth muscle. *Bayero Journal of Pure and Applied Sciences*. 2014;7(2):21-5. <https://doi.org/10.4314/bajopas.v7i2.5>
 14. Bagayogo M. Contrôle de qualité botanique des plantes des Médicaments Traditionnels Améliorés du Departement Medecine Traditionnel du Mali: USTTB; 2020.
 15. Bekro Y-A, Mamyrbekova J, Boua BB, Bi FT, Ehile EE. Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpinaceae). *Sciences & Nature*. 2007;4(2):217-25. <https://doi.org/10.4314/scinat.v4i2.42146>
 16. Singleton VL, Orthofer R, Lamuela-Raventos RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in enzymology*. 1999;299:152-78. [https://doi.org/10.1016/S0076-6879\(99\)99017-1](https://doi.org/10.1016/S0076-6879(99)99017-1)
 17. Kumaran A, Joel Karunakaran R. In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. *LWT - Food Science and Technology*. 2007;40(2):344-52. <https://doi.org/10.1016/j.lwt.2005.09.011>
 18. Abdel-Hameed E-SS. Total phenolic contents and free radical scavenging activity of certain Egyptian *Ficus* species leaf samples. *Food Chemistry*. 2009;114(4):1271-7. <https://doi.org/10.1016/j.foodchem.2008.11.005>
 19. Kim KS, Lee S, Lee YS, Jung SH, Park Y, Shin KH, et al. Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. *Journal of ethnopharmacology*. 2003;85(1):69-72. [https://doi.org/10.1016/S0378-8741\(02\)00338-0](https://doi.org/10.1016/S0378-8741(02)00338-0) PMID:12576204
 20. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology & medicine*. 1999;26(9-10):1231-7. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3) PMID:10381194
 21. Arts MJTJ, Sebastiaan Dallinga J, Voss H-P, Haenen GRMM, Bast A. A new approach to assess the total antioxidant capacity using the TEAC assay. *Food Chemistry*. 2004;88(4):567-70. <https://doi.org/10.1016/j.foodchem.2004.02.008>
 22. Sombié PEAD, Hilou A, Coulibaly AY, Tibiri A, Kiendrebeogo M, O.G. N. Brain protective and erythrocytes hemolysis inhibition potentials from galls of *Guiera senegalensis* JF Gmel (Combretaceae). *Journal of pharmacology and toxicology*. 2011;6(4):361-70. <https://doi.org/10.3923/jpt.2011.361.370>
 23. OCDE. Toxicité orale aiguë - Méthode par classe de toxicité aiguë. . Lignes directrices de l'OCDE pour les essais de produits chimiques 2001.
 24. Ouédraogo N, Lompo M, Sawadogo R, Tibiri A, Hay A-E, Koudou J, et al. Anti-inflammatory, analgesic and antipyretic activities of aqueous decoction of the leaves and roots bark of *Pterocarpus erinaceus* Poir. (Fabaceae) 2012. 286-92 p. <https://doi.org/10.1007/s10298-012-0732-z>
 25. Traoré KT, Ouédraogo N, Belemnaba L, Boly GAL, Kabré LMEW, Atchadé CB, et al. Anti-inflammatory and analgesic activities of extracts from *Balanites aegyptiaca* L. Delile (Balanitaceae) root bark: Plant used against liver diseases in Bukina Faso. *African Journal of Pharmacy and Pharmacology*. 2019;13(18):322-9.
 26. Ouédraogo S, Yoda J, Traore TK, Nitiema M, Sombie BC, Diawara HZ, et al. Production de matières premières et fabrication des médicaments à base de plantes médicinales. *International Journal of Biological and Chemical Sciences*. 2021;15(2):750-72. <https://doi.org/10.4314/ijbcs.v15i2.28>
 27. Ph.Eur. Pharmacopée européenne 10ème édition 10.0, 10.1, 10.2-edqm / conseil de l'europe 2019.
 28. Rivera LL. Etude de l'extraction de métabolites secondaires de différentes matières végétales en réacteur chauffé par induction thermomagnétique directe: Institut National Polytechnique (Toulouse); 2006.
 29. Faye PG, Ndiaye EM, Ndiaye B, Cisse OIK, Ayessou NC, Cisse M. Effet de la macération, de l'infusion et la décoction sur l'extraction aqueuse des polyphénols des feuilles séchées de *Combretum Micranthum*. *Afrique SCIENCE*. 2022;21(3):114-26.
 30. Dhanani T, Shah S, Gajbhiye NA, Kumar S. Effect of extraction methods on yield, phytochemical constituents and antioxidant activity of *Withania somnifera*. *Arabian Journal of Chemistry*. 2017;10:S1193-S9. <https://doi.org/10.1016/j.arabjc.2013.02.015>
 31. Bohui PSG, Adima AA, Niamké FB, N'Guessan JD. Etude comparative de trois méthodes d'extraction des flavonoïdes totaux à partir des feuilles de plantes médicinales: *Azadirachta indica* et *Psidium guajava*. *Journal de la Société Ouest-Africaine de Chimie*. 2018;46:50-8.

32. Tine Y, Sene M, Gaye C, Diallo A, Ndiaye B, Ndoeye I, et al. Combretum micranthum G. Don (Combretaceae): A Review on Traditional Uses, Phytochemistry, Pharmacology and Toxicology. Chemistry & biodiversity. 2024;21(5):e202301606. <https://doi.org/10.1002/cbdv.202301606> PMID:38353648
33. Ramadwa TE, Meddows-Taylor S. Traditional uses, pharmacological activities, and phytochemical analysis of Diospyros mespiliformis Hochst. ex A. DC (Ebenaceae): A review. Molecules (Basel, Switzerland). 2023;28(23):7759. <https://doi.org/10.3390/molecules28237759> PMID:38067488 PMCID:PMC10708241
34. Hawas UW, El-Ansari MA, El-Hagrassi AM. A new acylated flavone glycoside, in vitro antioxidant and antimicrobial activities from Saudi Diospyros mespiliformis Hochst. ex A. DC (Ebenaceae) leaves. Zeitschrift fur Naturforschung C, Journal of biosciences. 2022;77(9-10):387-93. <https://doi.org/10.1515/znc-2021-0291> PMID:35245970
35. Martínez-Pérez E, Juárez Z, Hernandez L, Bach H. Natural Antispasmodics: Source, Stereochemical Configuration, and Biological Activity. BioMed Research International. 2018;2018:1-32. <https://doi.org/10.1155/2018/3819714> PMID:30402474 PMCID:PMC6196993
36. Muanda FN. Identification de polyphénols, évaluation de leur activité antioxydante et étude de leurs propriétés biologiques. Université Paul Verlaine-Metz. 2010;238.
37. Foyzun T, Mahmud AA, Ahammed MS, Manik MIN, Hasan MK, Islam KM, et al. Polyphenolics with strong antioxidant activity from Acacia nilotica ameliorate some biochemical signs of arsenic-induced neurotoxicity and oxidative stress in mice. Molecules (Basel, Switzerland). 2022;27(3):1037. <https://doi.org/10.3390/molecules27031037> PMID:35164302 PMCID:PMC8840196
38. Bhol NK, Bhanjadeo MM, Singh AK, Dash UC, Ojha RR, Majhi S, et al. The interplay between cytokines, inflammation, and antioxidants: mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. Biomedicine & Pharmacotherapy. 2024;178:117177. <https://doi.org/10.1016/j.biopha.2024.117177> PMID:39053423
39. Unies N. Système Général Harmonisé de classification et d'étiquetage des produits chimiques (SGH). Quatrième édition révisée. New York et Genève. ST/SG/AC.10/30/Rev4. : Nations unies; 2011.
40. Muttaka A, Abdullahi L, Sani M. Toxicological Studies of the Aqueous Leaves Extracts of Combretum micranthum on Rats. 2016;12:167-71.
41. Lai FA, Erickson HP, Rousseau E, Liu QY, Meissner G. Purification and reconstitution of the calcium release channel from skeletal muscle. Nature. 1988;331(6154):315-9. <https://doi.org/10.1038/331315a0> PMID:2448641
42. Webb RC. Smooth muscle contraction and relaxation. Advances in physiology education. 2003;27(1-4):201-6. <https://doi.org/10.1152/advan.00025.2003> PMID:14627618
43. Schlemper V, Ribas A, Nicolau M, Cechinel Filho V. Antispasmodic effects of hydroalcoholic extract of Marrubium vulgare on isolated tissues. Phytomedicine. 1996;3(2):211-6. [https://doi.org/10.1016/S0944-7113\(96\)80038-9](https://doi.org/10.1016/S0944-7113(96)80038-9) PMID:23194972