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Original Article

EVALUATION OF THE EFFECT OF THE AQUEOUS EXTRACT OF THE RECIPE MADE FROM THE LEAVES OF *OCIMUM GRATISSIMUM*. LINN (LAMIACEAE) AND *TERMINALIA SUPERBA* STEAM BARK. ENGL (COMBREATACEAE) ON LOPERAMIDE-INDUCED CONSTIPATION

ELION ITOU ROMARIC DE GARDE[1,2,3*], BOUKONGO ROGER PAVEL[1], MAMBEKE HONO MAMBEKE[1], ETOU OSSIBI ARNAUD WILFRID[1,2], MORABANDZA CYR JONAS[4], ABENA ANGE ANTOINE[3]

[1]Animal Physiology Laboratory, Faculty of Science and Technology, Marien University Ngouabi, BP69, Brazzaville, Congo. [2]National Institute for Health Sciences Research (Scientific City of Brazzaville (Formerly ORSTOM), Route de l'Auberge de Gascogne (Chateau d'eau), Congo. [3]Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, University Marien Ngouabi, BP 69, Brazzaville, Congo. [4]Department of Natural Sciences, Ecole Normale Superieure, Marien University, Ngouabi Congo, B. P 69, Brazzaville, Congo

[*]Corresponding author: Elion Itou Romaric De Garde; [*]Email: romaricelion@gmail.com

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ABSTRACT

Objective: This study aimed to contributing to the promotion of the recipe made from the leaves of *O. grastissium* and the bark of *T. superba* used as a laxative.

Methods: Constipation was induced in rats by oral administration of loperamide (3 mg/kg body weight) for three days. The constipated rats were treated with the aqueous extract of the recipe (125, 250 and 500 mg/kg) for 7 d. In addition, the effect of the aqueous extract of the recipe was also evaluated on the secretion of prostaglandin E2, nitric oxide (NO), on the accumulation of intestinal fluid, the secretion of electrolytes as well as on some biochemical parameters.

Results: The results obtained show that the aqueous extract at the doses used significantly reduced the delay ($p<0.001$), significantly increased ($p<0.01$ and $p<0.001$) the frequency and quantity of stools excreted in rats made constipated by the loperamide. Similarly, the aqueous extract of the recipe stimulated a significant increase ($p<0.001$) in the secretion of prostaglandin E2, nitric oxide, water, ions such as K+, Na+, Ca2+and Cl- leading to intraluminal water retention and accumulation of intestinal fluid. Moreover, unlike castor oil, the aqueous extract of the recipe at the doses used does not cause any significant increase in biochemical parameters compared to control animals.

Conclusion: The results obtained show that the aqueous extract of the recipe has a laxative activity, which would be due to the chemical compounds highlighted. These results could justify the traditional use of this recipe.

Keywords: Constipation, Recipe, Loperamide, *Ocimum gratissimum Terminalia superba*

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INTRODUCTION

Constipation is a very common, often chronic, gastrointestinal disorder that affects adults [1, 2]. It arises from the inhibition of ion and fluid secretion, which is one of the most distressing side effects of opioids, for example, the analgesic treatment with lopéramide [3, 4]. The treatment of this involves the use of laxatives, which act in a different way on intestinal transit. However, conventional laxatives are only accessible to a privileged category of the population because of their high costs and the distance from pharmacies, which poses a real problem in the management of patients to improve their quality of life [5, 6]. Hence, many populations are forced to resort to the leaves and bark of medicinal plants presumed to have laxative properties for treatment. These plants can be used alone or in combination in a recipe where certain plants will potentiate the expected effect or serve as an excipient [7]. Medicinal plants have several pharmacological properties for example, antioxidant and anti-inflammatory. However, they can also have side effects when consumed in high concentrations, which will induce toxicity in different organs [8]. Therefore, toxicological, phytochemical, and pharmacological studies of medicinal plants must be carried out, in order to provide a coherent scientific justification for the traditional use of it [9]. Indeed, the leaves of *O. grastissium* and the steam bark of *T. superba* are respectively used in the treatment of infections of the upper respiratory tract (colds, coughs, bronchitis), ear infections, and stomach aches. Bark tea is prescribed as an anti-dysenteric to sterile women and against miscarriages [10]. In addition, previous studies carried out separately attribute to them respectively anti-malarial [11], anti-diabetic [12, 13] properties. *T. superba* is used in the southern and central regions of Cameroon in the treatment of diabetes and cardiovascular disorders [13, 14]. No scientific study has been carried out on the possible laxative effects of the recipe made from *O. grastissium* leaves and *T. superba* bark. Thus, in order to give a scientific basis to the traditional use of this recipe, we have set ourselves the objective of contributing to the promotion of the recipe made from the leaves of *O. grastissium* and the bark of *T. superba* used as a laxative.

MATERIALS AND METHODS

Plant materials

The leaves of *O. grastissium* and the steam barks of *T. superba were used*. Several plant leaves of the same species *O. grastissium* growing in the same place and steam barks of of *T. superba* were collected in July 2021 in the locality of Mossendjo, Department of Niari (Congo). Botanical identification of the plant material was done by Mousamboté, botanist systematist of Higher Normal School of Agronomy and Forestry (HNSAF) and confirmed at the herbarium of the National Institute for Research in Exact and Natural Sciences (NIRENS) in which a collected sample was compared to a reference samples n ° 8012 for *O. grastissium* and n °6025 for *T. superba* respectively. After identification, the plant material was dried at room temperature (27±50 °C) for 14 d in the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE), and then pulverized with a mortar. The powder obtained was used to prepare the aqueous extract of the recipe. The aqueous extract for the recipe of *O. grastissium* leaves and *T. superba* bark was prepared by maceration at 10 %. 12.25 g of *O. grastissium* leaf powder and 12.5 g of *T. superba bark* powder were mixed in 500 ml of distilled

water. Then, the mixture was left to macerate under a magnetic stirrer for 24 h. After filtration using cotton wool, the maceration obtained was evaporated at a temperature of 65 °C using an evaporator (Buchi R-100-11593665) in order to obtain a dry extract. The dry extract obtained was used for pharmacological tests.

Animals

Albino rats (100 to 150 g) and albino mices (15 to 30 g) of either sex aged 3 mo were used. These animals were provided to us by the animal house of the Laboratory of Pharmacodynamics and Experimental Physiopathology (L2PE) of the Faculty of Science and Technical of the Marien Ngouabi University. They were fed with a standard food and with running water. They were acclimatized during one week before experimentation and were housed under standard conditions (12 h light and 12 h dark) and at the temperature of 27±1 °C. The rules of ethics published by the International Association for the Study of Pain have been considered [15].

Evaluation of the effect of the aqueous extract on the onset, frequency and quantity of excretion of stools in non-constipated rats

It was assessed using the method reported by Mikhail *et al.*, (2015) [16]. This method consists in evaluating beforehand the properties of a plant supposed to be laxative. For this, five (5) groups of five (5) rats each were formed and treated orally with different doses of distilled water (negative control group 1: 1 ml/100g of distilled water); castor oil (positive control: 2 ml/rat) and the aqueous extract of the recipe (125, 250 and 500 mg/kg). After administration tests, the animals were placed in metabolic cages for observation. During this observation, the onset (the minimum time the animal takes to make stools), the frequency and the quantity of stool emission were evaluated for 8 h.

Evaluation of the effect of the aqueous extract of the recipe on loperamide-induced constipation

The method reported by Elion Itou *et al.*, (2023) [17] was used. Prior to the induction of constipation, the normals were observed for seven (7) days in the metabolite cages. During these seven days, they were fed standard food and water. Thus, food consumption, fluid intake, number of faecal pellets, water content of pellets, weight of pellets and twenty-four (24) hour weight gain were determined. Water content was calculated as the difference between the dry weight and the wet weight of the stool. After this observation, constipation was induced in all these animals by oral administration of loperamide hydrochloride (5 mg/kg body weight in 0.9 % sodium chloride) for three days. The same parameters as before were observed. Animals showing signs of constipation (presence of hard or dry stools) were selected and divided into five (5) groups of five (5) rats each treated for one week (07 d) with different doses of water distilled (negative control group: 1 ml/100g); castor oil (positive control: 2 ml/rat) and the aqueous extract of the recipe (125, 250 and 500 mg/kg). After oral administration of the products, the animals were placed individually in the metabolic cages for eight hours (8^{th}) for observation. Thus, food consumption, fluid intake, fecal pellet count, pellet water content, pellet weight, and twenty-four 24 h weight gain were determined for the duration of the experiment (07 d). After 7 d of treatment, the blood of each rat was taken by puncture in the retro-orbital sinus at the level of the eye, and put in dry tubes then centrifuged using a centrifuge (3000 thousand revolutions/30 min) in order to to measure some biochemical parameters: Alkaline phosphatas (ALP), Aspartate amino transferase (ASAT), ALAT (alanine-amino-transferase), Glycemia, Total cholesterol, LDH (lactate deshydrogenase), HDL (high-density lipoprotein), Creatinemia and Triglycerides. In addition, this blood was also used to measure prostaglandin E_2 and nitric oxide (NO).

Phytochemical profile of the aqueous extract of the recipe

Phytochemical tests of tube reactions were performed following the method reported by Wadood *et al.*, 2013 [18].

Statistical analysis

All values were expressed as mean±standard error of the mean (SEM). An analysis of variance using Excel version 2016 software followed by Student-Fischer t-test "t" was performed. The significance level was set at $p<0.05$

RESULTS

Effect of the aqueous extract of the recipe on the onset, frequency and quantity of sells excreted in non-constipated (normal) animals

Tables 1, 2 and 3 present the effect of the aqueous extract of the recipe on the onset, the frequency and the quantity of sells excreted in non-constipated rats. It appears from this study that the animals treated with castor oil (2 ml/rat) as well as the aqueous extract (125, 250, and 500 mg/kg) reduced significantly ($p<0.001$) the onset excreted sells compared to the control group. The onset are 360±00 (control group), 120.28±12.20 (castor oil), 15±0.44, 15.2±0.48 and 15.6±0.24 for aqueous extract of the recipe at the doses of 125, 250, and 500 mg/kg reespectly (table 1). Furthermore, castor oil (2 ml/rat) as well as the aqueous extract (125, 250, and 500 mg/kg) significantly ($p<0.001$) increase the frequency of excreted sells (table 1) and the quantity excreted sells (table 2) compared to the control group. 8 h later, the frequencies excreted sells are: 00±00, 0.81±0.03, 0.51±0.03, 0.68±0.08 and 0.89±0.11 respectively for distilled water (control), castor oil as well as for the aqueous extract of the recipe used (125, 250, and 500 mg/kg) (table 2). At 8 h, the quantities excreted sells are: 00±00, 2.87±0.00, 2.90±0.02, 2.96±0.82 and 2.97±0.05 respectively for distilled water (control), castor oil as well as for aqueous extract of the recipe used (125, 250, and 500 mg/kg) (table 3).

Table 1: **Effect of the aqueous extract of the recipe on the onset of stool emission in non-constipated rats**

Treatment	Dose	Onset excreted sells (Mn)
Control group	0.5 ml/100 g	360±00
Castor oil	2 ml/rat	120.28±12.20***
	(125 mg/kg)	15±0.44***
Aqueous extract	(250 mg/kg)	15.2±0.48***
	(500 mg/kg)	15.6±0.24***

Table 2: **Effect of the aqueous extract of the recipe on the frequency of excreted sells in non-constipated rat**

Treatment	Doses	Frequency of excreted sells			
		2h	4h	6h	8h
Control group	0.5 ml/100g	00±00	00±00	00±00	00±00
Castor oil	2 ml/rat	3.33±0.38***	2.31±0.07***	3.01±0.16***	0.81±0.03 ***
	125 mg/kg	3.50±0.27***	2.39±0.09***	2.75±0.07***	0.51±0.03 ***
Aqueous extract	250 mg/kg	3.71±0.21***	2.46±0.12***	2.89±0.02***	0.68±0.08 ***
	500 mg/kg	3.96±0.31***	2.49±0.21***	2.97±0.07***	0.89±0.11 ***

Each value represents the mean±SEM (n=5 per group) of frequency. ***p <0.001, Significant different (Student t-test) versus control group.

Table 3: **Effect of the aqueous extract of the recipe on the quantity of excreted sells in non-constipated rats**

Treatment	Dose	Quantity of excreted sells (g)			
		2h	4h	6h	8h
Control group	0.5 ml/100g	00±00	00±00	00±00	00±00
Castor oil	2 ml/rat	1.86±0.04***	2.23±0.13***	2.78±0.09***	2.87±0.01***
Aqueous extract	125 mg/kg	1.83±0.06***	2.25±0.10***	2.77±0.15***	2.90±0.02***
	250 mg/kg	1.85±0.06***	2.36±0.12***	2.78±0.22***	2.96±0.82**
	500 mg/kg	1.88±0.05***	2.48±0.14***	2.81±0.12***	2.97±0.05***

Each value represents the mean±ESM (n= 5 per group). ***p <0.001, Significant different (Student t-test) versus control group.

Effect of the aqueous extract of the recipe on constipated rats

Table 4 presents the effect of loperamide on food consumption, water intake, number of faecal pellets, water content of the pellets, weight of the pellets and weight gain three days before administration of the test products compared with the non-constipated animals. It appears from this study that loperamide induced constipation in all non-constipated (normal) animals, which resulted in a significant decrease (p <0.01 and p<0.001) of these parameters, with the exception of the weight which increases significantly (p <0.001) compared to the control group (Cg). Furthermore, the oral administration of the aqueous extract (125, 250 and 500 mg/kg) of the recipe to constipated rats shows a significant reduction in onset of excreted sells (p<0.001) (table 5), a significant increase (p<0.001) in the frequency of excreted sells (table 6) as well as the quantity of excreted sells during the 8 h of experimentation (table 7). In addition, oral administration of castor oil, the aqueous extract of the recipe made from the leaves of *O. grastissium* and the bark of *T. superba* at increasing doses (125, 250 and 500 mg/kg) significantly increase food consumption, water intake, number of faecal pellets, water content of pellets, weight of pellets and reduced weight gain of constipated animals treated during the seven days by those having received distilled water (table 8).

Table 4: **Effect of loperamide on food consumption, water consumption and fecal properties in untreated constipated rats**

Paramètres	Controle normal	Rats constipes
Food consumption	25.69±0.40	40.36±2.30 ***
water intake	17.89±1.68	9,68±0.65 **
Number of faecal pellets	21.57±1.42	6.63±0.44 **
Water content of pellets	3.04±0.39	0.52±0.53**
Weight of stool excreted	2.15±0.36	0.47±0.06 ***
Body weight	231.62±2.47	256.45±0.99***

Each value represents the mean±ESM (n= 5 per group) of sells parameters. **p<0.01; ***p<0.001, Significant different (Student t-test) versus control group.

Table 5: **Effect of the aqueous extract of the recipe on onset excreted sells in constipated rats treated for 7 d**

Treatement	Dose	Onset excreted sells (Mn)
loperamide+control group	0.5 ml/100 g	360±00
loperamide+castor oil	2 ml/rat	120.28±13.58***
loperamide+aqueous extract	125 mg/kg	10.05±0.50***
	250 mg/kg	10.74±0.30***
	500 mg/kg	10.63±0.25***

Each value represents the mean±ESM (n= 5 per group) of onset. ***p<0.001, Significant different (Student t-test) versus control group.

Table 6: Effect of the aqueous extract of the recipe on the cumulative of frequencies of excreted sells in constipated rats during seven days of experimentation

Treatment	Dose	Frequency of excreted sells			
		2h	4h	6h	8h
Loperamide+control group	0.5 ml/100g	00±00	00±00	00±00	00±00
Loperamide+Castor oil	2 ml/rat	5.42±2.42***	2.42±0.42***	2.44±0.12***	1.32±0.26***
Aqueous extract+loperamide	125 mg/kg	6.08±2.27***	2.48±0.12***	2.42±0.04***	1.33±0.02***
	250 mg/kg	5.54±2.47***	2.51±0.27***	2.26±0.17***	1.31±0.03***
	500 mg/kg	5.42±2.35***	2.51±0.05***	2.28±0.03***	1.30±0.12***

Each value represents the mean±ESM (n= 5 per group) of frequency. ***p<0.001, Significant different (Student t-test)

Table 7: **Effect of the aqueous extract of the recipe on the cumulative of quantities of excreted sells in constipated rats during seven days of experimentation**

Treatment	Dose	Quantity of excreted sells			
		2h	4h	6h	8h
Loperamide+control group	0.5 ml/100g	00±00	00±00	00±000	00±00
Loperamide+castor oil	2 ml/rat	2.60±0.69***	3.40±0.22**	3.69±0.39***	4.13±0.57***
Aqueous extract+loperamide	125 mg/kg	2.61±0.10***	3.43±0.35***	4.27±0.19***	4.70±0.25***
	250 mg/kg	2.59±0.22***	3.57±0.24***	4.34±0.17***	5.42±0.15***
	500 mg/kg	2.59±0.37***	3.46±0.21***	4.26±0.39***	4.43±0.38***

Each value represents the mean±ESM (n= 5 per group) of frequency. ***p<0.001, Significant different (Student t-test) versus control group.

Table 8: Effects of the aqueous extract of the recipe on fecal parameters in constipated and treated rats during seven days of experimentation

Sells parameters	Constipated+distilled water	Constipated+castor oil	Constipated rats+recipe aqueous extract		
			125 mg/kg	250 mg/kg	500 mg/kg
Food consumption	26.60±1.53	17.07±1.47 **	14.09±1.53 ***	15.10±1.44 ***	14.68±1.24 ***
water intake	18.10±0.70	12.57±0.44 ***	12.29±0.57 ***	12.65±0.45 ***	12.88±0.62 ***
Number of faecal pellets	00±00	282±3.11***	268±1.90 ***	274±1.62 ***	289±1.49 ***
Water content of pellets	00±00	12.06±0.67 ***	12.83±1.66 ***	13.25±1.12 ***	10.41±0.26 ***
Weight of stool excreted	00±00	10,70±0.53 ***	7.51±0.68 ***	8.24±0.38 ***	8.93±0.33 ***
Body weight	222.02±4.32	173.40±2.61 ***	189.05±0.53 ***	186.65±0.61 ***	187.39±0.15 ***

Each value represents the mean±ESM (n= 5 per group) of sells parameters. ***p<0.001, Significant different (Student t-test) versus control group.

Effect of the aqueous extract of the recipe on the secretion of prostaglandin E_2 and nitric oxide (NO) in constipated rats

Table 9 shows the effect of the aqueous extract of the recipe for *O. grastissium* leaves and *T. superba* steam bark on the secretion of prostaglandin E_2 and nitric oxide (NO). This results in a significant increase (p<0.001) in prostaglandin E_2 concentrations (24.27±2.13; 13.88±0.10; 15.43±0.51 and 16.03±0.32; p<0.001), as well as nitric oxide (0.98±0.00; 0.77±0.09; 0.73±0.06 and 0.74±0.02; p<0.001) in animals treated with castor oil and the aqueous extract of the recipe at doses (125, 250 and 500 mg/kg) compared to control group.

Table 9: Effect of the aqueous extract of the recipe on stimulation of prostaglandin E2 and nitric oxide (NO) secretion

Treatment	Doses	Prostaglandin E_2	Ntric oxide (NO)
Control group	0.5 ml/100g	3.56±0.37	0.01±0.00
Castor oil	2 ml/rat	24.27±2.13***	0.98±0.00***
Aqueous extract	125 mg/kg	13.88±0.10***	0.77±0.09 ***
	250 mg/kg	15.43±0.51***	0.73±0.06 ***
	500 mg/kg	16.03±0.32***	0.74±0.02 ***

Each value represents the mean±ESM (n= 5 per group) of sells parameters. ***p<0.001, Significant different (Student t-test) versus control group.

Effect of the aqueous extract of the recipe on the biochemical parameters of constipated rats treated for 7 d

Table 10 presents the results of the effect of the aqueous extract of the recipe made from *O grastissium* leaves and *T. superba* steam bark on biochemical parameters in rats made constipated and treated for 7 d. It appears that the aqueous extract of the recipe of *O grastissium* leaves and *T. superba* steam bark at doses of 125, 250 and 500 mg/kg of body weight does not lead to any significant increase in biochemical parameters compared to control animals. However, a significant increase (p<0.01 and p<0.001) is observed, in particular in the activities of alkaline phosphatase, ALT, AST and LDH in animals having received castor oil.

Phytochemical of the aqueous extract of the recipe

The results of the phytochemical analysis of the maceration of the aqueous extract of the recipe. This phytochemical analysis reveals the presence of several chemical constituents including anthraquinones, alkaloids, flavonoids, terpenoids, tannins, reducing sugars, oses and mucilages. On the other hand, the saponosides are absent in the maceration of the recipe.

Table 10: Effects of the aqueous extract of the recipe on biochemical parameters after daily administration for 7 d

Biochemical parameters	Treatment for a week (7 d)				
	Control group (0.5 ml/100g)	Castor oil (2 ml/rat)	Aqueous extract (125 mg/kg)	Aqueous extract (250 mg/kg)	Aqueous extract (500 mg/kg)
ASAT UI/l	12.78±0.43	24.82±0.71* **	12.12±0.69 ns	13.32±0.66 ns	13.34±0.32 ns
ALAT UI/l	17.81±1.40	35.86±0.86** *	16.71±2.45 ns	17.70±2.44 ns	17.32±1.93 ns
ALP UI/l	32.05±1.11	49,42±1.78** *	29.55±2.12 ns	30.47±2.55 ns	30.02±3.75 ns
Creatinin (mg/l)	0.77±0.05	1.30±0.16**	0.74±0.04 ns	0.72±0.04 ns	0.78±0.03 ns
Glucose (g/l)	0.66±0.02	0.79±0.04 ns	0.66±0.08 ns	0.67±0.04 ns	0.69±0.03 ns
Triglycérides (mg/l)	35.67±2.45	49.81±0.99***	35.12±1.93 ns	35.36±1.25 ns	36.29±1.66 ns
LDH (UI/l)	0.73±0.07	3.63±0.65**	0.71±0.00 ns	0.74±0.03 ns	0.73±0.01 ns
HDL (mg/l)	0.86±0,03	12.72±0.58 ***	0.59±0.03 ns	0.56±0.15 ns	0.57±0.04 ns
TC (mg/l)	31.84±1.64	48.38±0.65* **	31.60±0.35 ns	30.92±1.72 ns	31.35±1.96 ns

Each value represents the mean±ESM. (n= 5 per group). **p<0.01; ***p<0.01 Significant different (Student t-test) versus control group; ns (p>0.05; no signicant different (Student t-test) versus control group. ASAT: aspartate aminotransferase; ALAT: alanine-amino-transferase; HDL: high-density lipoprotein; LDH: lactate deshydrogenase; ALP: alkaline phosphatase, TC: Total cholesterol.

DISCUSSION

The present study was initiated to determine the phytochemical profile of the aqueous extract of the recipe of *O. grastissium* leaves and *T. superba* barks and to evaluate the effect of the aqueous extract of the recipe on constipation. Prior to this, the effect of aqueous extract from the recipe of *O. grastissium* leaves and *T. superba* steam bark was evaluated in normal rats to find out whether the extract would behave either as a laxative or as an antidiarrheal. The results obtained show that the aqueous extract of the recipe of the leaves of *O. grastissium* and the bark of *T. superba* at the doses used significantly reduced the onset of excreted sells, increased the frequency of excreted sells and the quantity of excreted sells like castor oil compared to the control. Castor oil causes diarrhea due to its active metabolite, ricinoleic acid [19], which stimulates peristaltic activity in the small intestine, leading to changes in the electrolyte permeability of the intestinal mucosa. Its action also stimulates the release of endogenous prostaglandins and

platelet-activating factor [20, 21] which promote vasodilation, smooth muscle contraction and mucus secretion in the small intestine, resulting in diarrhea [22, 23]. Observation of the results obtained suggests a laxative effect of the aqueous extract of the recipe. Thus, the laxative effect of the aqueous extract of the recipe of *O. grastissium* leaves and *T. superba* bark was evaluated on constipation induced by loperamide. Indeed, loperamide, known as an antidiarrheal, is an opioid receptor agonist acting on the μ-opioid receptors in the myenteric plexus, which in turn decreases the smooth muscle tone of the intestinal wall. In addition, it promotes the activation of presynaptic opioid receptors present in the enteric nervous system and thus inhibits the release of acetylcholine, decreasing peristalsis and thus increasing intestinal transit time [23]. Loperamide-induced constipation is therefore considered a model of spastic constipation [24]. The results of this study show that loperamide caused constipation in all animals, which resulted in a significant decrease ($p < 0.01$ and $p < 0.001$) in food consumption, water intake, the number of faecal pellets, water content of faecal pellets, weight of faecal pellets followed by a significant increase ($p < 0.001$) in weight gain three days before administration of the test products compared to normal animals. The observed reduction in the number, weight and water content of fecal pellets after drug treatment indicated constipation in rats. These results are also similar to those of Elion Itou *et al.*, (2023) [17] in the study on the laxative effect of the aqueous extract of the leaves of *Crossopteryx febrifuga* benth (Rubiaceae). On the other hand, the aqueous extract of the recipe (125, 250 and 500 mg/kg) administered to constipated rats significantly increases food consumption, water intake, the number of faecal granules, the water content of the granules, the weight of pellets and reduced the weight gain of the constipated rats treated during the seven days compared to the control group. In addition, oral administration of the aqueous extract (125, 250 and 500 mg/kg) to constipated rats showed a significant decrease in onset of excreted sells ($p < 0.001$), a significant increase ($p < 0.01$ and $p < 0.001$) of the frequency and quantity of excreted sells as castor oil compared to the control group. The increase in the water content of the stool, frequency and quantity of excreted sells, and decrease of the onset excreted sells suggest a laxative effect of the aqueous extract of the recipe of the leaves of *O. grastissium* and the steam barks of *T. superba,* which could act like castor oil used as reference laxative. To verify this hypothesis, the effect of the aqueous extract of the recipe at the doses used was evaluated on the production of prostaglandin E_2 and nitric oxide, which are two very important intermediates involved in the induction mechanism of diarrhea from castor oil. The results obtained show that the aqueous extract of the recipe at increasing doses causes a significant increase in the concentration of prostaglandin E_2 and the nitric oxide secreted compared to the controls treated with distilled water. Indeed, castor oil can directly activate macrophages and increase the production and secretion of prostaglandin E_2. Prostaglandin E_2 can act on colonic mucosal epithelial cells as a paracrine factor and decrease the expression of aquaporin-3 in the colon [25]. The results of this study suggest that compounds that decrease aquaporin-3 expression in the colon or activate macrophages may exhibit a laxative effect by increasing fecal water content [25]. In addition to prostaglandin E2, castor oil can also promote the release of nitric oxide (NO) and the activation of adenylyl cyclase with a consequent increase in the concentration of cAMP. The consequent increase in the concentration of cAMP results in the stimulation of peristaltic activity in the intestine, thus modifying the permeability of the membrane, reducing the activity of the Na+K+ATPase pump, thus decreasing the absorption of Na+and K+, causing the accumulation of these electrolytes and water in the intestinal lumen [26]. Its cathartic action is due to the accumulation of water in the intestine [27]. These results suggest that the aqueous extract of the recipe would act like castor oil (the standard molecule).

Since constipation is widespread, often chronic, affecting adults [2], prolonged treatment with the aqueous extract of the recipe from *O. grastissium* leaves and *T. superba* steam bark could lead to an increase in the activities of certain enzymes, hence the need to carry out an analysis of certain biochemical parameters. Indeed, the biochemical analyzes carried out showed a non-significant decrease in alkaline phosphatase (ALP), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), ALAT (alanine-amino-transferase), creatinine (Créat), HDL-cholesterol (high-density lipoprotein-cholesterol), glycaemia and cholesterol (CT) in animals having received the aqueous extract of the recipe compared to controls treated with distilled water. Elevated serum alkaline phosphatase levels are known to be associated with the risk of coronary heart disease and total mortality as are triglycerides [28, 29]. Similarly, the aqueous extract of the recipe causes a decrease in blood sugar, creatinine, total cholesterol, and HDL cholesterol. The decrease in these three biochemical parameters suggests that this aqueous extract of the recipe could reduce the risk of obesity, cardiovascular disease and kidney damage [30, 31]. Similar effects have been obtained with other plant extracts, such as *Monodora myristica* [32], *Cassipourea congoensis* [33]. However, a significant increase ($p < 0.01$ and $p < 0.001$) of the following enzymatic parameters is observed: alkaline phosphatase (AL), lactate dehydrogenase (LDH), aspartate aminotransferase (ASAT), ALAT (alamnineaminotransferase), creatinine (Creat), total cholesterol (TC) and HDL in animals given castor oil. Transaminases or amino-transferases are tissue enzymes that catalyze the transport of alpha-amino radicals from alanine and aspartic acid to alpha-ketoglutaric acid. Transaminases are present in the liver, but also in muscle and ASTs in the kidney, pancreas, and other tissues. They are synthesized in the cytoplasm of the cells of these organs and released into the circulation when these cells are damaged [34]. These enzymes increase in case of myopathy, rhabdomyolysis or myocardial infarction and AST, particularly in case of haemolysis. ALTs are more specific for liver damage, but ASTs are somewhat more sensitive [35]. In the case of this study, it could be that the aqueous extract of the recipe has a hepatoprotective action at the doses used.

Phytochemically, the laxative effect of the aqueous extract of the recipe of *O. grastissium* leaves and *T. superba* steam bark could be due to the presence of secondary metabolites contained in the aqueous extract of the recipe known to have laxative properties such as anthraquinones [7-36], flavonoids [38], alkaloids, saponosides [39]. Anthraquinone and its active derivatives such as anthraquinone glycosides stimulate peristalsis of the small intestine and increase peristaltic movements of the colon. The latter are hydrophilic and reduce the absorption of water in order to have a fluid faecal bolus [40]. They, therefore, prevent the formation of lumpy stools. Anthraquinone is used as a laxative or purgative. The presence of phytoconstituents like trapezoids, sterols, flavonoids, phenolic compounds, tannins and alkaloids [41] have already been shown to be responsible for the laxative activities of plants.

CONCLUSION

This study shown that the aqueous extract of the recipe formulated by the leaves of *O. grastissium* and the steam bark of *T. superba* possesses laxative properties. This laxative effect could be achieved by increasing the production of prostaglandins E2 and nitric oxide, as would the castor oil used as a reference molecule. In addition, the aqueous extract has hepatoprotective properties. The laxative effect observed could be explained by the presence of secondary metabolites highlighted in this extract. With a view to implementing a drug based on this improved traditional recipe, additional studies are necessary.

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AUTHORS CONTRIBUTIONS

Elion Itou RDG and Mambeke H. M carried out the experimental studies, Boukongo R. P collected plant samples in his department; Etou Ossibi AW and Morabandza C. J participated in the writing of the document. Abena AA supervised the study. All authors have read and approved the final manuscript of this article.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interes

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