

BIOCHEMICAL CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF A COMPOSITE EXTRACT FROM THREE EDIBLE PLANTS

¹Kouadio Jean-Baptiste N'Guessan and ²Yao Marie-Claire Ahou

¹Nutrition and food security laboratory, UFR of food sciences and technologies, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

²Biology and Health Laboratory, UFR Biosciences, Félix Houphouët-Boigny University (UFHB), 22 BP 582 Abidjan 22, Ivory Coast.

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Corresponding author: Kouadio Jean-Baptiste N'Guessan

Nutrition and food security laboratory, UFR of food sciences and technologies, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract: Chronic diseases with an inflammatory component are the major cause of mortality in the world. The treatment of inflammation is generally based on chemical drugs. Nutritionists recommend the use of food plants that might have antiinflammatory effects. So, the phenolic compounds, chemical composition, acute toxicity, in vitro and in vivo anti-inflammatory activities and pain-relieving activity of a recipe extract based on the leaves extract of three food plants (Ceiba pentandra, Ipomoea batatas and Spinacia oleracea) were evaluated. The results showed that the yield of extraction was 20.7 % and revealed the presence of polyphenols (821.6 mg/100 g) and flavonoids (99.8 mg/100 g). The chemical composition gave: proteins 0.023 %, lipids 0.011 %, ash 0.013 %, fibers 0.011 % and assimilables carbohydrates 0.026 %. The acute toxicity test did not cause any mortality, nor any sign of toxicity at the dose of 5000 mg/Kg/bw. In vitro anti-inflammatory test showed that the plants extract, at the concentrations of 150 and 250 µg/mL had an inhibitory effect, respectively of 65.63% and 86.97%. For in vivo anti-inflammatory test, at the doses of 400 and 800 mg/Kg/bw, the extract has an optimum inhibition of 79.95% and 53.62%, on a mice paw edema, at the fourth hour after injection. The pain test showed inhibitions of 94.74%; 98.25% and 100% at the respective doses of 300, 600 and 1200 mg/Kg/bw. The results therefore suggest that the recipe of the three food plants has an anti-inflammatory activity, certainly due to the presence of polyphenols and flavonoids.

Keywords: Anti-inflammatory activity, Ceiba pentandra, Ipomoea batatas, Spinacia oleracea, Phenolic compounds.

INTRODUCTION

Inflammation is manifested, among other things, by the appearance of clinical signs such as redness, heat, swelling and pain in the case of several diseases (Labonté, 2015; Zerbato, 2010). It induces the activation of receptors of the innate immune system, which leads to the release of pro-inflammatory chemical mediators (cytokines, serotonin, bradykinin) (Labonté, 2015;

Tiendrebeogo, 2012). The etiology of inflammation is generally exogenous (wound, burn), but can also be endogenous (pathological situations such as sickle cell disease, type 2 diabetes, gout, cancer, etc.) (Sene et al., 2016). In fact, chronic diseases with an inflammatory component are the main cause of death in the world (Dembélé, 2020). According to the World Health Organization, 36 million people die each year worldwide from these diseases. These deaths, 80% of which occur in low- and middle-income countries, represent 3 out of 5 deaths, or 63% of all global deaths from all causes combined. In Côte d'Ivoire, deaths from these diseases (sickle cell disease, type 2 diabetes, CVD, cancer) represent 31% of all deaths (PNMNT, 2019). Inflammation and eating habits are intimately linked (Labonté, 2015). Indeed, foods that are too sweet, too salty, too fatty and the lack of fruit and vegetables in food promote the development of inflammation (Lefief -Delcourt & Proust-Million, 2019). Treatment of inflammation is based on nonsteroidal (NSAID) and steroidal (AIS) anti-inflammatory drugs. In 2007, an American study revealed that among 1261 athletes tested, 63% consumed drugs, including 64% NSAIDs (LaiCheung-Kit, 2018). In the long term, these active molecules have harmful side effects on the body. With risks of gastrointestinal toxicity such as peptic ulcer, perforation or stenosis. There are also kidney risks such as acute kidney failure and sometimes heart complications (Kouadio et al., 2021). In addition, the cost and availability of this treatment are a constraint for rural populations. Nutritionists and dieticians propose as an alternative the use of food plants rich in antioxidant compounds in the treatment of inflammatory diseases (Taiba et al., 2017). Indeed, since antiquity man has used food and medicinal plants provided by his natural environment to ensure his well-being. (Taiba et al., 2017). These food plants contain secondary metabolites (i.e. flavonoids, polyphenols) with biological activities antioxidant, appropriate in the treatment of inflammatory reactions which are associated with the development of multifactorial metabolic disorders (Meradji & Merrakchi, 2020). Food plants also have nutritional properties, due to the presence of primary metabolites, such as proteins, carbohydrates, lipids and essential amino acids. Edible plants are inexpensive and available in the local market (Bouquet & Debray, 1974). It would be interesting to identify local food plants that have anti-inflammatory activity, that can replace non-steroidal anti-inflammatory drugs and glucocorticoids, while meeting the nutritional needs of the body (Grimm & Parel, 2019). So, the main objective of the present study is to evaluate the antiinflammatory activity of a recipe based on the leaves of three food plants: *Ceiba pentandra*, *Ipomoea batatas* and *Spinacia oleracea*. To achieve this goal, it specifically involved to determine the phenolic compounds, chemical composition, acute toxicity, in vitro and in vivo anti-inflammatory activities and pain-relieving activity of a recipe extract based on the leaves of three food plants (*Ceiba pentandra*, *Ipomoea batatas* and *Spinacia oleracea*) were evaluated.

MATERIAL AND METHODS

Plant material

The plant material used in this work consisted of the leaves of *Ceiba pentandra*, *Ipomoea batatas* and *Spinacia oleracea*. *Ceiba pentandra* leaves were collected in the tropical rainforest of Yabi kan, in the department of Akoupé, located at 142 km from Abidjan, in Côte d'Ivoire. Those of *Ipomoea batatas* and *Spinacia oleracea* were purchased from market gardeners in Akoupé. Then, they were packaged

and transported to the national floristic center (CNF) of the Félix Houphouët-Boigny University in Abidjan, where they were identified by an expert in botanical systematics. The identified leaves were then sent to the Central Laboratory of Nangui Abrogoua University in Abidjan.

Preparing the recipe extract

The leaves were washed, cut and then dried separately, away from the sun, at room temperature. They were then reduced to powder using an electric grinder (Retdch RM 200). The shredded material of each plant species (100 g) was mixed in a stainless-steel jar to make up the leaf recipe. Three hundred grams (300g) of recipe powder were dissolved in 3 liters of water and then boiled for 8 minutes. The decoction obtained was wrung out using a square of white cotton cloth. The solution was successively filtered 3 times on absorbent cotton and once on 3 mm filter paper. The filtrate obtained was evaporated in a Venticell type oven at 50° C, for 48 hours. The refined brown powder (decocted or concentrated) obtained constitutes the extract of the recipe. It was stored in the refrigerator at 5° C in an airtight jar.

Animal material

The animals used in our experiments are mice (*Mus musculus*). These animals were obtained at the animal facility of the National Superior School, of the Félix Houphouët Boigny University. These animals were composed of females and males, a total of 66 mice. They were 8 weeks old. The mice were nulliparous, non-pregnant with a mean body mass of 21g. All procedures were approved by the ethics committee of the Félix Houphouët-Boigny University and in accordance with the principles of the scientific ethics committee of biology for the use of laboratory animals for tests experimental (Aworet-Samseny *et al.*, 2011). As part of this work, they were used for acute toxicity test, anti-inflammatory tests and the pain test.

Analysis of the nutritional value of the extract of the recipe Protein content

The crude protein content was determined from total nitrogen using the Kjeldhal method (AOAC, 1990). One gram (1 g) of extract from the recipe was heated to 400 °C for 150 min in the presence of a pinch of the catalyst mixture (Selenium + potassium sulfate (K₂SO₄)) and 20 mL of sulfuric acid (H₂SO₄) at 95%. The mineralizate obtained was made up to 60 mL with distilled water. To this volume, 50 mL of 40% (w/v) sodium hydroxide were added, before being brought to the boil in a LEGALLAIS type distiller. The ammonia that was released was trapped in a measuring cup containing 10 mL of the 40% (w/v) acid-base mixture mixed indicator (methyl red + bromocresol green) at pH 4.4-5.8. Nitrogen was measured using a decimolar solution of sulfuric acid.

Fat content

The lipid content was determined using the en method (AOAC, 1990) using Soxhlet as an extractor. Ten grams (1 g) of extract from the recipe were placed in a cellulose extraction cartridge and plugged in with cotton. The cartridge was then introduced into the Soxhlet reservoir and oil extraction was carried out using a solvent ebb and flow system using 300 mL of n-hexane. The heating was carried out using heating caps. After 7 h of extraction, the solvent (n-hexane) was recovered using a rotary evaporator (HEIDOLPH, Germany). The flask, initially tared and containing oil, was weighed to determine the mass of oil extracted.

Ash content

The method used for the determination of ash was described by AOAC (1990), which consists of incinerating a sample until white ash is obtained. Five grams (2 g) of sample previously dried in the oven and then crushed were weighed in a porcelain incineration capsule of known mass. The capsule containing the sample was placed in a muffle furnace (PYROLABO, Germany) and incinerated at 550 °C for 12 h. After removing the capsule from the muffle oven and then cooling in a desiccator, it was weighed again. The ash content was expressed as a percentage by mass.

Crude fiber content

The determination of the crude fiber content was carried out according to the method of AOAC (1990). Two grams (2 g) of sample were added to a flask, then 50 mL of 0.25N sulfuric acid was added. The resulting mixture was homogenized and boiled for 30 min under reflux condenser. After 30 min, 50 mL of 0.31N sodium hydroxide (NaOH) was added to the contents, was filtered through Whatman paper and the residue was washed several times with hot water until the alkalis were completely removed. The residue was oven dried at 105°C for 8 h. After cooling in a desiccator, the residue was weighed and then incinerated in an oven at 55° C. for 3 h. After cooling, the ash obtained was weighed.

Assimilables carbohydrates content and energy values

The assimilables carbohydrates and the energy value were determined according to the calculation method recommended by FAO (2002). This method takes into account, the protein, fat, ash and fiber contents and, the energy value.

Dosage of total phenols of the recipe extract

The Folin-Ciocalteu method of (Singleton et al., 1999) was used to measure total phenols. The reagent, consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) was reduced during the oxidation of phenols, into a mixture of blue oxides of tungsten and molybdenum. The color produced was proportional to the amount of phenols present in the plant extracts. One (1) mL of methanolic extract was added to a test tube. To the contents of the tube was added one (1) mL of Folin-Ciocalteu's reagent. The tube was left to stand for 3 minutes then 1 mL of 20% (w/v) sodium carbonate solution was added to it. The contents of the tube were made up to 10mL with distilled water. The tube was placed in the dark for 30 minutes and the OD reading was taken at 725 nm against a blank. A standard range established from a stock solution of gallic acid (1 mg/mL) under the same conditions as the test made it possible to determine the quantity of phenols in the sample.

Dosage of flavonoids of the recipe extract

The determination of the flavonoids was carried out according to the method described by (Meda et al., 2005) the principle of which is as follows: the flavonoids react with aluminum chloride in the presence of potassium acetate to give a complex of yellow color whose intensity is proportional to the quantity of flavonoids present in the medium. A volume of 0.5mL of methanolic extract was introduced into a test tube. To the contents of the tube were successively added 0.5 mL of distilled water, 0.5 mL of 10% (w/v) aluminium chloride, 0.5 mL of 1 M potassium acetate and 2 mL of distilled water. The tube was left to stand in the dark for 30 min and the optical density (OD) was read at 415 nm against

a blank. A standard range, established from a quercetin stock solution (0.1 mg/mL) under the same conditions as the test, made it possible to determine the quantity of flavonoids in the sample.

Assessment of the acute toxicity of the recipe extract

The acute toxicity study was carried out according to the European guideline of the OECD 423 (OECD, 2001). This is a method by class of acute toxicity allowing to assess the dose levels that can lead to the death of a laboratory animal, to know the symptoms of acute poisoning as well as the circumstances of death. Nulliparous and non-pregnant female mice were used to conduct the experiment. They have been marked for identification. They were then fasted for 16 h and given only water. Two batches of five (5) mice were composed. The extract was dissolved in physiological water and then administered in a single dose to the mice by gavage with a volume relative to the weight of the mouse. Batch 1 (control) received 1 mL of distilled water throughout the duration of the experiment. The second batch received the extract at a single dose of 5000 mg/kg body weight (BW). The animals were then observed individually, at least once during the first 30 min and regularly during the first 24 h after the treatment. Four hours after access to food, the animals were again observed for possible toxicological signs. Daily during 14 days, signs such as tremor, convulsion, salivation, diarrhea, lethargy, sleep and coma were noted. The skin, hair, eyes and mucous membranes, as well as the respiratory system were also explored. During the 14 days of observation, animals were weighed daily for the first week and every two days for the second week at the same hours.

***in vitro* anti-inflammatory activity of the recipe extract**

in vitro anti-inflammatory activity of the recipe extract was carried out according to the protein denaturation inhibition method described by (Lavanya et al., 2010). The method consisted in preparing four solutions:

- 1- The test solution (0.5 mL) composed of 0.45 mL of the aqueous solution of Bovine Serum Albumin (BSA) 5% and 0.05 mL of aqueous solution of recipe extract with a concentration of 250 µg/mL;
- 2- The control test solution (0.5 mL) composed of 0.45 mL of the aqueous solution of BSA 5% and 0.05 mL of distilled water;
- 3- The product control solution (0.5 mL) composed of 0.45 mL of distilled water and 0.05 mL of aqueous solution of recipe extract with a concentration of 250 µg/mL;
- 4- The standard test solution (0.5 mL) composed of 0.45 mL of the aqueous solution of BSA 5% and 0.05 mL of the reference product Diclofenac sodium with a concentration of 250 µg/mL.

All the solutions were adjusted to pH 6.3 with an HCl solution (1N), the samples were incubated at 37°C for 20 min, then the temperature was increased to 57°C for 3 min. After cooling the tubes, 2.5 mL of phosphate buffer saline solution (pH 6.3) was added to the cooled solutions and the absorbance was measured by the UV-visible spectrophotometer at 416 nm.

***In vivo* anti-inflammatory activity of the recipe extract**

The induction of the edema of the left hind paw of the mouse by carrageenan (1%) was carried out according to the method of Winter et al. (2012). Six groups of 5 mice each were made up as follows:

- Batch 1 or normal control: received distilled water;

- Batch 2 or untreated control: received 0.1mL of 1% carrageenan and distilled water;
- Batch 3 or batch treated with Diclofenac: received 0.1 mL of carrageenan and 10 mg/Kg bw of Diclofenac;
- Batch 4 or batch treated with the recipe extract at a dose of 200 mg/Kg bw;
- Batch 5 or batch treated with the recipe extract at a dose of 400 mg/Kg bw ; - Batch 6 or batch treated with the recipe extract at a dose of 800 mg/Kg bw. The mice weighed between 15 and 30 g, they were fasted for 24 hours before the experiment. Measurements of the diameter of the left hind paw of each mouse were made and the means per group calculated. The animals were then force-fed with the extract, and one hour later, edema was induced with 0.1 mL of the 1% carrageenan solution, injected cutaneously at the level of the plantar aponeurosis of the left hind paw of each mouse. The measurements of the diameter of the induced edema were made at one hour, 2 hours, 3 hours, 4 hours and 5 hours after the injection of carrageenan. (Anupama et al., 2012). The measurements were made using a digital caliper (Bukhari et al., 2013). The extent of edema was evaluated by determining the mean percentage of increase in the thickness of the mouse paw. Anti-inflammatory activity was also assessed by calculating the percent of inhibition of edema.

Painkiller effect of the recipe extract by injection of 0.6% acetic acid

Pain was induced in mice, intraperitoneally, using a dilute solution of 0.6% acetic acid as described by Koster et al. (1959). Five groups of five mice were each treated as follows:

- Negative control batch treated with distilled water at a dose of 0.5 mL/100 Kg bw;
- Positive control batch treated with paracetamol at 100 mg/Kg bw;
- Batch 3 treated with the recipe extract at a dose of 300 mg/Kg bw;
- Batch 4 treated with the recipe extract at a dose of 600 mg/Kg bw; - Batch 5 treated with the recipe extract at a dose of 1200 mg/Kg bw.

One hour after administration of the products by gavage, acetic acid at a dose of 10 ml/kg bw was injected into the animals by the intraperitoneal route. After injection of the acetic acid solution, the mice were placed individually in cages and a latency time of 5 min was observed. The number of abdominal twists performed by each mouse over the next 20 min was counted (Elion Itou et al., 2014). The activity is expressed as a percentage of pain inhibition for each group treated at the different doses.

Statistical analysis of results

The results were expressed as a mean accompanied by standard errors on the mean (Mean $\pm$ SEM). The graphical representation of the data was carried out using the GraphPad software Prism 5. As for the two-factor variance analysis, it was carried out by ANOVA with repeated experiments and the Dunnett test enabled us to determine the significant differences at the threshold 5 %.

RESULTS AND DISCUSSION

Yield and chemical composition of the recipe extract.

Table I presents the results of the extraction yield, the chemical composition and the phenolic compounds in the recipe extract. After the extraction, a fine hygroscopic powder of brown color was obtained. The yield of this extraction was 20.7 ± 2.1 %. The polyphenol content of the recipe extract was 821.6 ± 0.5 mg/100 g and the flavonoid content was 99.8 ± 0.1 mg/100 g. As for the chemical

composition, the contents of proteins, lipids ash, fibers, and carbohydrates were respectively $0,023 \pm 0,002$ (%), $0,011$ (%), $0,013 \pm 0,003$ (%), $0,011 \pm 0,002$ and $0,026 \pm 0,001$ (%). The energy value was $0,254$ Kcal/100 g.

Table I: Yield, phenolic compounds and chemical composition of the recipe extract.

Settings	Values
Yield	20.7 ± 2.1 (%)
Polyphenols	821.6 ± 5.1 mg/100g
Flavonoids	99.8 ± 0.1 mg/100g
Humidity	9 ± 0.02 (%)
Proteins	$0,023 \pm 0,002$ (%)
Lipids	$0,011 \pm 0,003$ (%)
Ash	$0,013 \pm 0,003$
Fibers	$0,011 \pm 0,002$ (%)
Assimilables carbohydrates	$0,026 \pm 0,001$ (%)
Energetic value	$0,254$ Kcal/100 g

The decoction was chosen as the mode of extraction, because it is the procedure most used by the local populations for the preparation of herbal medicines and for cooking food. According to Markham (1982) and Jones & Kinghorn (2005), the aqueous extracts contain flavonoids, polyphenols and tannins. However, prolonged decoction could lead to the degradation of thermolabile molecules (Cheaib et al., 2018). Polyphenols are important in nutrition and health because they have antioxidant and anti-inflammatory properties (Gardner et al., 2000). Flavonoids have several antiinflammatory activities, analgesic, antioxidant properties (Ghedira, 2005). Polyphenols and Flavonoids play an important role against the development of inflammatory diseases such as sickle cell disease, cardiovascular diseases, cancers and type II diabetes (Williamson, 2017; Oulai et al., 2014; Hodge & Sterner, 1943). The chemical composition of the extract of the recipe showed that the protein, lipids, ash, fibers, carbohydrates contents and energetic value were very low.

Evaluation of the acute toxicity of the extract from the recipe.

Table II presents the results of the administration of the dose of 5000 mg/Kg bw the aqueous extract of the recipe of plants. Regular observation for 14 days showed no signs of toxicity (weight loss, salivation, drowsiness, coma, morbidity) and no mortality in the treated mice (weight loss, salivation, drowsiness, coma, morbidity). During the observation, the animals having presented a slight agitation (they scratched and moved a lot) only in the first minutes compared to those of the control group. However, this is not part of the signs of toxicity. In addition, there was a slight non-significant weight gain in mice tested compared to mice in the control lot.

Table II: Evolution of the weight of the mice after the administration of the extract of the recipe.

Products	Dose administered (mg/Kg bw)	Average mouse weight (g)		Weight gain (g)
		Day 1	Day 14	

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Distilled water	Witness	18.313±2.92	20.793 ± 1.63	2.48 ± 1.29 ^a
Extract from the recipe	5000	14.24± 1.80	15.86± 2.90	1.62± 1.1

Values are means ± standard deviation. In the same column, the means having an identical letter in common are not significantly different ($p < 0.05$) according to Dunnett's test.

The single dose of 5000 mg/kg bw showed no evidence of toxicity or mortality in treated mice. This means that its lethal dose of 50 (LD₅₀) was greater than 5000 mg/kg bw. According to the scale of (Hodge and Sterner, 1943), the recipe extract is classified as Category 5 and considered a non-toxic substance.

***In vitro* anti-inflammatory effect on protein denaturation of recipe extract**

The results of the *in vitro* anti-inflammatory test of the recipe extract were presented in Figure 1. Diclofenac sodium (a pharmaceutical anti-inflammatory) was used as the reference molecule. *In vitro* anti-inflammatory tests showed that the plants recipe extract and Diclofenac at concentrations of 150 and 250 µg/mL had an inhibitory effect on protein (BSA) denaturation. Protein denaturation is a characteristic of inflammatory diseases (Kada, 2018). Inhibition percentages were 65.63% (at 150 µg/mL) and 86.97% (at 250 µg/mL) for the recipe extract.

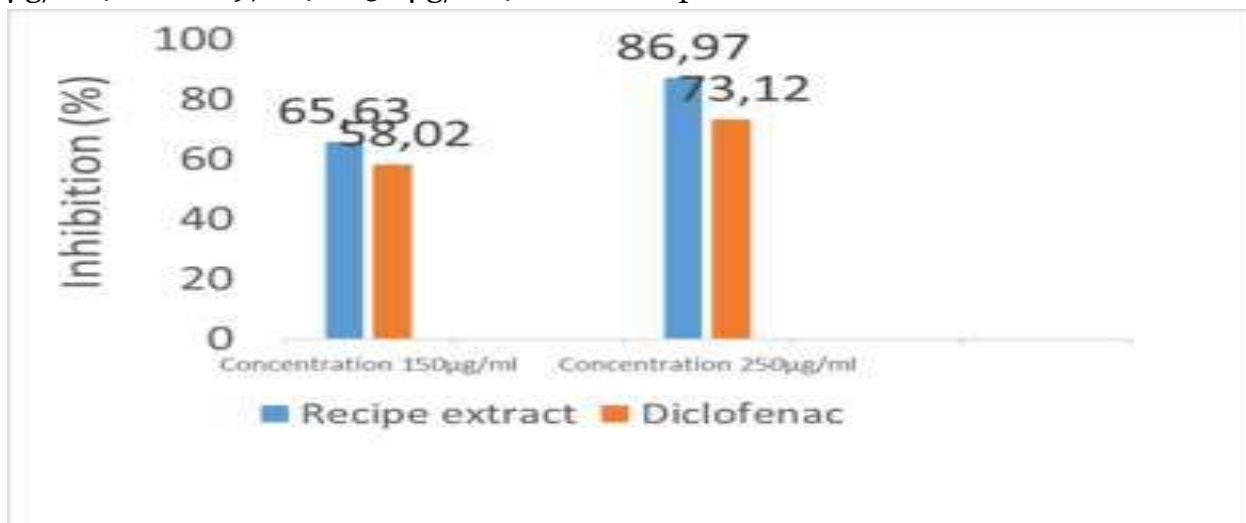


Figure 1: Inhibition of BSA denaturation.

The percentages of inhibition were 58.02% (at 150 µg/mL) and 78.12% (at 250 µg/mL) for Diclofenac. This showed that the anti-inflammatory effect of the recipe extract was superior to that of Diclofenac. This could be due to the presence of antioxidants like flavonoids and polyphenols in the recipe extract. According to Ndibualonji et al. (2016), the leaves of *Ceiba pentandra* contain flavonoids, saponins and tannins that have antioxidant capacity and could help to explain its healing properties.

***In vivo* anti-inflammatory activity of recipe extract on edema induced on the left leg of the mice by 1% carrageenan**

The results of the *in vivo* anti-inflammatory test were presented in Figure 2. The recipe extract at a dose of 200 mg/kg bw and diclofenac (10 mg/kg bw) showed no inhibition of edema at the second and third hour. At the fourth hour, there was an inhibition of edema of 16.96% (with 200 mg/kg bw of recipe extract) and 30.72% (with 10 mg/kg bw of diclofenac). As for the doses of 400 and 800 mg/Kg bw, the recipe extract showed inhibition of the edema at the second and the third hour. And at the fourth hour there was an effective inhibition of edema of 79.95% (with 400 mg/kg bw of recipe extract) and 53.62% (with 800 mg/kg bw of recipe extract).

The anti-edematous effect of the recipe extract at doses of 400 and 800 mg/Kg bw was greater than that of Diclofenac during the experiment. The extract acts in a dose-dependent manner. The dose of 400 mg/Kg bw is the optimal dose.

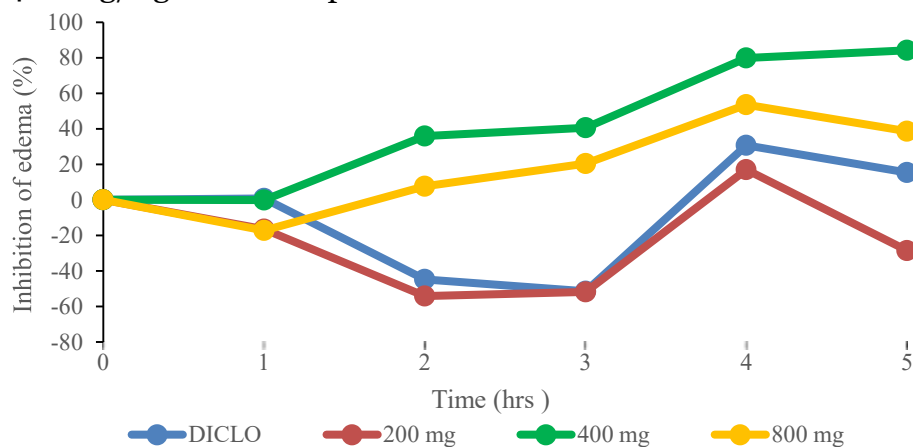


Figure 2: Edema inhibition effect of the recipe extracts (200; 400 and 800 mg/Kg/bw) and Diclofenac. Inflammation is a mechanism that involves the release of mediators by activating mast cells, these will release serotonin and histamine (Kada, 2018). This will lead to a chain of events producing chemotactic factors like the chemokine. These promote the migration of leukocytes to the inflammatory site (Kada, 2018).

These results suggest that the recipe extract had anti-inflammatory effect that opposes the action of endogenous pro-inflammatory mediators. This action would be exerted more on cyclooxygenase, enzyme responsible for the synthesis of prostaglandins (Perez-Guerrero et al., 2001). This work showed that the recipe extract based on the three food plants contained an appreciable amount of secondary Metabolites such as polyphenols and flavonoids which may explained this anti-inflammatory effect.

Painkiller effect of the recipe extract by injection of 0.6% acetic acid

The results of the analgesic test presented in Table 3 showed that administration of the 300, 600 and 1200 mg/Kg bw doses of the recipe extract exerted a protective effect against acetic acid pain. In addition, this inhibitory effect on abdominal contortions is significantly higher than that of the

reference molecule Paracetamol. This inhibitory effect increases in intensity when the dose of the extract increases. The highest percentage inhibition is 100% at a dose of 1200 mg/Kg bw.

Table 3: Painkiller effect of the extract of the recipe on the abdominal contortions induced in mice after the injection of acetic acid.

Products and doses (mg/Kg)	Medium contortions	% inhibition
TN Distilled water (0.5mL/100 g)	28.5±12.02	-
TP Paracetamol (100 mg/Kg/bw)	24.5±3.54	14.04
RE (300 mg/Kg/bw)	1.5 ± 0.71*	94.74
RE (600 mg/Kg/bw)	0.5 ± 0.71*	98.25
RE (1200 mg/Kg/bw)	0 ± 0*	100

Values are means ± standard deviation. TN: Negative control, TP: Positive control, ER: Recipe extract. * $p < 0.05$; $n = 5$: significant difference compared to the Paracetamol reference molecule.

The method of abdominal contortions induced by acetic acid was used to highlight the analgesia of the recipe extract with an effect independent of inflammation. The occurrence of abdominal contortions under the effect of acetic acid involves the action of local peritoneal receptors (Ouédraogo et al., 2012) as well as the release of mediators such as prostaglandins (PGE₂α, PGF₂α) and cytokines (TNF-α, IL-1β, IL-8) (Bentley et al., 1983; Negus et al., 2006). The recipe extract reduced the number of abdominal contortions in a dose-dependent and significant manner compared to the control mouse. The analgesic effect of the recipe extract at the smallest tested dose of 300 mg/Kg bw was greater than that of Paracetamol. This suggests that the analgesic effect of the extract of the recipe may be related to the inhibition of the release of chemical mediators (Perez-Guerrero et al., 2001; Vane; 1972).

CONCLUSION

The objective of this work was to evaluate the anti-inflammatory activity in vitro and in vivo of the extract of a recipe based on three food plants (*Ceiba pentandra*, *Spinacia oleracea* and *Ipomoea batatas*). The results showed that the yield of extraction per decoction was 20.7%. The results highlighted the presence of polyphenols and flavonoids known for their antioxidant roles. The chemical composition showed that the recipe extract was low in ash, protein, lipid and fiber. The acute toxicity test of the extract showed that the recipe extract was not toxic. The results of the in vitro antiinflammatory test showed that the recipe extract at 150 and 250 µg/mL inhibited protein denaturation better than diclofenac. As for the doses of 400 and 800 mg/Kg bw of the recipe extract, it had an increasing inhibitory action throughout the treatment; from the 2nd to the 5th hour. The inhibition percentages at the 4th hour were 79.95% for the 400mg dose and 53.62% for the 800 mg/Kg bw dose. Doses of 400 and 800 mg/Kg bw had higher inhibition than Diclofenac. The results of the analgesic test showed that the extract inhibited the pain. This inhibition was significantly higher than that of paracetamol. In view of these results, the extract of the recipe of three plants, could be used as a vegetal anti-inflammatory and would act in the treatment of inflammatory diseases such as sickle cell disease, diabetes and cancer.

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