Evaluation of antidiarrheal effect of the aqueous extract of the leaves of *Chromolaena odorata* L (King and Robinson)

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**ABSTRACT**

This study aimed to evaluate the antidiarrheal effect of the aqueous extract of the leaves of *C. odorata* (400 and 800 mg/kg). The antidiarrheal effect was evaluated on the diarrhoea induced by the castor oil, the charcoal test (intestinal transit time) and on the accumulation of the intestinal fluid induced by the castor oil (Enteropooling). The results obtained show that the aqueous extract at the doses used significantly decrease (p<0.001) the frequency of emission, the quantity and the onset of appearance of the faces induced by the castor oil. The aqueous extract of *C. odorata* (400 and 800 mg/kg) does not decrease significantly the intestinal transit (p>0.05) but on the other hand significantly decrease (p<0.01) the accumulation of the fluid in the intestine induced by the castor oil. In conclusion the aqueous extract of *C. odorata* (400 and 800 mg/kg) has an antidiarrheal effect who could be explained by interference with the mechanisms of secretion of the electrolytes. These results would justify the use of plant on the traditional treatment of the diarrhoea.

**Keywords:** antidiarrheal effect, Castor oil, intestinal transit, enteropooling, *Chromolaena odorata*

**INTRODUCTION**

In developing countries, people in rural areas often resort to traditional medicine to treat their diseases, including problems related to diarrhea. Indeed, diarrhea is a major cause of mortality and infant morbidity. Diarrheal diseases cause an estimated 1.8 million deaths each year in the world, of which 90% are children under five years, most of whom live in developing countries 1. 17% of children admitted to pediatrics die of diarrhea. They are the third leading cause of death for infectious diseases of all ages 2. 3 and the fifth leading cause of premature death in the worldwide 4. During the last 15 years, research has been undertaken to discover new drugs. It is becoming increasingly clear that plants can be a source of cheaper new products, especially for developing populations, and effective against diarrhea 5. For example, WHO in the Africa region encourages African countries to undertake research on medicinal plants and to promote their use in health care systems 5. *C. odorata* (Asteraceae) one of the medicinal plants much used in the American traditional herbal, Asian and African for the treatment of several pathologies. In Congo, it is used as a disinfectant and healing wounds 7. A leaf decoction is used to treat colds, flu, asthma, fever 8, skin infections, conjunctivitis 9, 10, diabetes and malaria 11, diarrhea 12. The phytochemical study of the aqueous extract revealed the presence of saponins, alkaloids, glycosides-cardiotonics, steroids, tannins and flavonoids 12. Previous pharmacological work on this plant shows that it has several pharmacological properties such as: analgesic and anti-inflammatory 14, antibacterial 15, 16, antioxidant 17 and antiulcer 18. This study aims to evaluate the antidiarrheal effect of the aqueous extract of leaves of *C. odorata*.

**MATERIAL AND METHOD**

**Plant material**

The leaves of *C. odorata* collected in Brazzaville were used. 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 botanical laboratory of Research Institute in Exact and Natural Sciences (RIENS) of Brazzaville where the samples of *C. odorata* was compared with the reference samples of the herbarium at No. 1183, July 1965. Plant material were dried and pulverized with a mortar. The aqueous extract of leaves of *C. odorata* was prepared by decoction. 250 g of powder of dry *C. odorata* leaves were mixed in 2500 ml of distilled water in a stainless steel container and kept at a temperature of 90°C for 30 minutes. After cooling, the extract was filtered through Whatman number 1 and stored at 4°C until use.
water. The mixture was boiled for 15 minutes. After cooling and filtration, the filtrate obtained was concentrated on a double boiler (60 °C). The concentrate obtained was preserved to evaluate the antidiarrheal effect.

Animal material
Albino rats (200 to 250 g) and albino mice (20 to 30 g) of either sex obtained from the Faculty of Science and Technology of Marien NGOUABI-University were used. They were fed with a standard feed and water ad libitum. 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 19 have been considered.

Castor oil induced diarrhoea in rat
The method reported by Elion Itou, (2018b) 20 was used. The animals were divided into groups of 5 rats each. The different doses of the aqueous extract of leaves of Codorata (400 and 800), loperamide (reference molecule, 10 mg/kg) and physiological saline (control, 0.5 ml/100g) were administered orally to groups, one hour prior castor oil administration (2 ml/rat). After castor oil administration, the animals were placed in metabolism cages to evaluate the frequency, the quantity of the faeces emitted as well as the onset of appearance of the diarrheal faeces (soft or liquids). The frequency and the faeces quantity were noted at 2, 4 and 6 hours after administration of castor oil.

Intestinal transit
Intestinal transit was determined by the charcoal method 20. The various doses of the aqueous extract of leaves of Codorata (400 and 800), loperamide (reference molecule, 10 mg/kg), physiological saline (control, 0.5 ml/100g) were administered orally to groups, one hour prior of 10% charcoal (10 ml/kg). 30 minutes after administration of the charcoal, the animals were sacrificed by cervical dislocation, the abdomen opened, the small intestine removed and placed on blotting paper. The small intestine is inspected, the intestinal contents of each group were collected in a tube and sent to the laboratory to determine the concentrations of Na + (sodium), K + (potassium) ions using a flame photometer (Micro Touch Biochemistry Analyser).

Statistical Analyze
All values were expressed as mean ± ESM. Analysis of variance followed by Student-Fischer t test “t” was performed. The significance level was set at p<0.05

RESULTS
Effect on diarrhea induced by castor oil
The administration of castor oil caused the stools to be shed. The results obtained show that loperamide and the aqueous extract at the doses used significantly reduce (p <0.001) the emission frequency as well as the quantity of faeces excreted (Table 1 and 2) during the 6 hours of observations compared to the control group. However, maximum decreases are observed the first two hours when loperamide and the aqueous extract at the dose of 800 mg/kg cause almost constipation of the animals. In contrast, loperamide and the aqueous extract at the doses used significantly reduce (p <0.001) the onset of the first diarrheal faeces appearance compared to the control group (Table 3). The onset of the first diarrheal faeces is 65.80 ± 1.01; 259.44 ± 1.44; 170.12 ± 0.79 and 224.04 ± 1.02 min respectively for physiological saline, loperamide and aqueous extract at doses of 400 and 800 mg/kg.

Enteropooling
This study involves assessing the net quantity of fluid accumulated in the small intestine. The various doses of the aqueous extract of leaves of Codorata (400 and 800), loperamide (reference molecule, 10 mg/kg) and distilled water (control, 0.5 ml/100g) were administered orally to groups, one hour prior administration of castor oil (2 ml/rat) 21. 2 hours after castor oil, the rats were sacrificed by cervical dislocation. The small intestine was removed and weighed (P1). Subsequently it was emptied of its contents then and weighed again (P2) and its length (L) measured. The difference between the weights divided by the length gives the net quantity (Q) of the fluid accumulated:

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Q = \frac{P1 - P2}{L}
\]

The intestinal contents of each group were collected in a tube and sent to the laboratory to determine the concentrations of Na + (sodium), K + (potassium) ions using a flame photometer (Micro Touch Biochemistry Analyser).

Table 1: Effect of aqueous extract of C. odorata on the frequency of fecal excretion

| Treatment     | Doses         | Frequency of faeces |
|---------------|---------------|---------------------|
|               | 2H            | 4H                  | 6H                  |
| Control group | 0.5 mL/100 g  | 14.4±1.07           | 18.6±0.6            | 20.6±0.67           |
| Loperamide    | 10 mg/kg      | 00±00***            | 1.16±0.21***        | 2.26±0.18***        |
| C. odorata     | 400 mg/kg     | 0,54±0.04***        | 5.6±0.22***         | 7.82±0.09***        |
|               | 800 mg/kg     | 00±00***            | 3.37±0.11***        | 4.48±0.18***        |

Each value represents the mean ± ESM; ***p<0.001 (Student t-test), versus control
Table 2: Effect of aqueous extract of C. odorata on faeces amount

| Treatment       | Doses          | Amount of faeces (g) | Inhibition (%) |
|-----------------|----------------|----------------------|----------------|
|                 | 2H       | 4H       | 6H       | 2H | 4H | 6H |
| Control group   | 0.5 mL/100 g | 6.72±0.18 | 8.84±0.24 | 10.16±0.09 | /  | /  | /  |
| Loperamide      | 10 mg/kg  | 0.00±0.00*** | 0.63±0.07*** | 1.63±0.40*** | 100 | 92.87 | 83.95 |
| C. odorata      | 400 mg/kg | 0.21±0.18*** | 3.84±0.60*** | 4.52±0.07*** | 96.75 | 56.56 | 55.51 |
|                 | 800 mg/kg | 0.00±0.00*** | 1.57±0.13*** | 2.68±0.03*** | 100 | 82.23 | 74.11 |

Each value represents the mean ± ESM; ***p<0.001 (Student t-test), versus control group

Table 3: Effect of aqueous extract of C. odorata on the onset of fecal excretion

| Treatment       | Doses          | Onset (Min) |
|-----------------|----------------|-------------|
| Control group   | 0.5 mL/100 g  | 65.80±1.01  |
| Loperamide      | 10 mg/kg      | 259.44±1.44*** |
| C. odorata      | 400 mg/kg     | 170.12±0.79*** |
|                 | 800 mg/kg     | 224.04±1.02*** |

Each value represents the mean ± ESM; ***p<0.001 (Student t-test), versus control group

Effect on intestinal Transit

The results of the effect of the aqueous extract of the leaves of C. odorata on intestinal transit are shown in figure 1. They show that loperamide significantly reduces (p < 0.001) intestinal transit compared to the control group (physiological water). However, the aqueous extract at the doses used did not significantly decrease intestinal transit (p> 0.05) compared with the control group. The intestinal transit values are 88.95 for control group; 31.35 for loperamide (reference molecule); 85.59 and 77.59 for the aqueous extract at the respective doses of 400 and 800 mg / kg.

Table 4: Effect of the aqueous extract of C. odorata on intestinal excretion of Na+ and K+ in mice

| Treatment       | Doses          | Na+ (mmol) | K+ (mmol) | Ratio (N+/K+) |
|-----------------|----------------|------------|-----------|---------------|
| Control group   | 0.5 mL/100 g  | 192.69     | 20.58     | 9.36          |
| Loperamide      | 10 mg/kg      | 89.001     | 21.53     | 4.13          |
| C. odorata      | 400 mg/kg     | 764.69     | 9.65      | 79.24         |
|                 | 800 mg/kg     | 758.04     | 18.43     | 41.13         |
Effect on intestinal fluid accumulation (Enteropooling)

The results of the effect of the aqueous extract of *C. odorata* leaves on intestinal fluid accumulation are shown in figure 2. They show that loperamide and the aqueous extract (800 mg / kg) decrease significantly (p <0.01) fluid accumulation in the intestine compared to the control group. However, no significant decrease (p> 0.05) was observed with the aqueous extract at 400 mg/kg. The masses of the fluid collected are 10.17 for control group; of 4.18 for loperamide, 10.02 for the 400 mg/kg of aqueous extract and 4.81 mg for the 800 mg/kg of aqueous extract. Moreover, the control, loperamide and the aqueous extract at the doses used (400 and 800 mg / kg) eliminate more Na + ions than K + ions (ratio >1).

Figure 2: Effect of the aqueous extract of *C. odorata* on intestinal fluid accumulation ***p<0.001 (Student t-test), versus control group; ns= no significant (p>0.05) test versus control group.

**DISCUSSION**

Oral administration of castor oil caused diarrhea in rats. This has resulted in an increase in the frequency of emission and the amount of faeces excreted which are two important parameters in the definition of diarrhea. In fact castor oil contains ricinoleic acid which induces irritation and inflammation of the intestinal mucosa, leading to the release of prostaglandins which, in turn, modify the mucous fluid and the transport of electrolytes, thus preventing the reabsorption of NaCl and water leading to a hypersecretory response and diarrhea. In fact, ricinoleic acid stimulates peristaltic activity in the small intestine and modifies the permeability of electrolytes (Na, K) by inhibiting the intestinal activity Na/K ATPase. Inhibition of intestinal activity Na/K ATPase, reduces normal fluid absorption by activation of adenylate cyclase. In this study, the aqueous extract of *C. odorata* (400 and 800 mg/kg) significantly reduced the faeces frequency emission; the amount of faeces excreted and delayed the onset of diarrhea induced by castor oil such as loperamide (reference molecule). Indeed, loperamide is one of the most widely used and best known antidiarrheal agents because of the absence of central effects in adults and its preferential binding to the μ and δ receptors of intestinal tissue. It antagonizes castor oil-induced diarrhea and its action is due to its antisecretory and anti-motility properties. Loperamide reduces intestinal motility by direct effect on the circular and longitudinal muscles of the intestinal wall. The fact that the extract is resistant to castor oil-induced diarrhea suggests that the aqueous extract at the doses used (400 and 800 mg / kg) would act as the loperamide used as the reference molecule. In addition, the reduction of motility and gastrointestinal secretions is one of the mechanisms by which many antidiarrheal agents act. Therefore, in this study, the effect of the aqueous extract was evaluated on the intestinal transit as well as on the accumulation of intestinal fluid induced by castor oil. The results obtained show that the aqueous extract of leaves of *C. odorata* does not significantly decrease (p> 0.05) the intestinal transit in contrast to loperamide (p <0.001) compared to the control group. This result suggests that the antidiarrheal effect observed would not pass an acceleration of intestinal transit. In addition, studies conducted on enteropooling (accumulation of intestinal fluid) revealed that the aqueous extract of *C. odorata* significantly inhibits the accumulation of intestinal fluid (enteropooling) induced by castor oil with a reduction in weight and the volume of intestinal contents. From these results observed with the aqueous extract, it can be said that the reduction of the intestinal fluid accumulation would be due to a stimulation of the absorption of electrolytes of the intestinal lumen comparable to the inhibition of hypersecretion. According to different physiopathological conditions of diarrhea, hypermotility characterizes diarrhea where the secretory component is not the responsible factor. It is possible that the extract may reduce diarrhea by increasing the reabsorption of electrolytes and water or by inhibiting the induction of intestinal fluid accumulation. However, it is known that prostaglandins stimulate gastrointestinal motility and secretion of water and electrolytes. The mechanism related to this would be associated with the dual effects of intestinal motility on both the transport of water and electrolytes (decreased absorption of Na + and K +) than on the intestinal mucosa. In this study, it was...
demonstrated that the aqueous extract of C. odorata at the doses used excrete more Na⁺ than K⁺ compared to the control and loperamide, which seems to confirm our hypothesis on the probable mechanism of the observed antidiarrheal effect. Other authors have already demonstrated the antidiarrheal effect of plant extracts. Indeed, these authors demonstrated the antidiarrheal effect of the aqueous extract (400 and 800 mg/kg) of the stem bark of Ceiba pentandra. The phytochemical study carried out previously had shown the presence of saponins, alkaloids, flavonoids, cardiotonic-heterosids, steroids and terpenoids as well as tannins. It was reported by various researchers that tannins, saponins and flavonoids can be responsible for antidiarrheal actions.

CONCLUSION

The objective of this work was to evaluate the antidiarrheal effect of the aqueous leaf extract of C. odorata. It appears from this study that the aqueous extract of C. odorata has an antidiarrheal effect. This effect could be achieved by reducing intestinal secretions and not by increasing intestinal transit. These results could explain the traditional use of C. odorata in the treatment of diarrhea.

REFERENCES

1. Gazaban M, Duffour J, Fabbro-Peray. Santé publique, 5e édition ; 2005 : p 242
2. WHO, 2011. Statistiques sanitaires mondiales.WHO, 2011 p171
3. Assogba A. L., Ehou E., Maiga M. F., NGueta Niamké E. E., Randremana R. V., Sehounou J., Seukap L. Initiation contre les maladies diarrhéiques et entériques en Afrique : une contribution à la lutte contre le choléra. Médecine d’Afrique Noire. 2012 : p7.
4. WHO, 2014. Statistiques sanitaires mondiales, Genève (Suisse) ; 2014 :p 12
5. Bashwira S. et Kahindo M. Screening phytochimie et évaluation de l’effet des extraits aquareux de quelques plantes médicinales sur E. coli, Sh. Flexineri et Sh dysenteriae type I dans le cahier de CERUK ; 1996, 27:4-10.
6. WHO, Comité régional de l’Afrique. Promouvoir le rôle de la médecine traditionnelle dans le système de Santé : Stratégie de la région africaine, rapport de la direction régionale 50ème session Burkinà Faso du 28 au 02 septembre 2000
7. ISS. Bissangou M.F et Ouamba J.M. Valorisation chimique de quelques plantes médicinales utilisées en pays Krobou (Aghville, Côte d’Ivoire). Sciences & Nature. (2009); 6(1):1-15.
8. Bouda H. Effect of essential oils from leave of Ageratum conyzoides, Lantana camara and Chromolaena odorata on the mortality of Sitopitus zeamais (Coleoptera, Cucurbitonidae). Journal of Stored Products Research. 2001; 37(2):103-109.
9. Agou S., Phytochemical investigation of Nigerian medicinal plants used in the treatment of skin disease. Thesis M. Sc. University de Ile, Nigeria. Journal of Crude Drug Research. 1980; 23: 253-256.
10. Irobi O.N., Antibiotic properties of ethanol extract of Chromolaena odorata (Asteraceae). International Journal of Pharmacognosy. 1997; 35(2):111-115.
11. Ayensu E. S., Medicinal plants of West Africa. References Publications Inc. Algonac, Michigan; 1972, p162.
12. Nguessam K. Screening phytochimiques de quelques plantes ibovenées utilisées en pays Krobou (Aghville, Côte d’Ivoire). Sciences & Nature. (2009); 6(1):1-15.
13. Elion Itou R. D. G, Mayela Nkouka S. H, J. Gouollaly Tsiba, Kassé D, Etou Ossibi A.W and Abena A. A. Antulcerogenic and Antioxidative effects of aqueous extract of Chromolaena odorata L. (King and Robinson). African Journal of Pharmacy and Pharmacology. 2013; 7(1):217-223
14. Elion Itou R. D. G, Etou Ossibi A. W, Epa C., Neondé Ntandou G. F, Bokia C. B, Ouamba J. M, and Abena A. A. Anti-inflammatory and analgesic effects of leaves of Chromolaena odorata L. (King and Robinson). African Journal of Pharmacy and Pharmacology. 2017; 11(17):217-223
15. Erchike CA, Mbanga Sassa A, ABBA A, Nynoubé E. Évaluation in vitro de l’activité antibactérienne de cinq plantes de la pharmacopée traditionnelle de l’Adamaoua (cameroun). Cameroun J. Exp. Biol. 2011; 7(1):22-27.
16. Agban A, Koffi AG, Eyana KA, Kokou T, Komban B, Kossi K, Koffi A. Evaluation des activités antimicrobiennes de Tridax procumbens (Asteraceae), Jatropha multifida (Euphorbiaceae) et de Chromolaena odorata (Asteraceae). Eur. Sci. J. 2013; 9(36):287-290.
17. Vijayaraghavan K, Mohamed Ali S, Maruthi R. Studies on Phytochemical Screening and Antioxidant Activity of Chromolaena Odorata and Annonas squamosa. International Journal of Innovative Research in Science, Engineering and Technology. 2013; 2 (12):2319-8753
18. Elion Itou R. D. G, Mayela Nkouka S. H, J. Gouollaly Tsiba, Kassé D, Etou Ossibi A.W and Abena A. A. Antulcerogenic and Antioxidative effects of aqueous extract of Chromolaena odorata L. (King and Robinson). World Journal of Pharmaceutical and Life Sciences. 2018a; 4(2):47-52
19. Bokia C. B, Ouedraogo L, Yakeri S, Amar P., Kibadi N. Evaluation in vitro de l’activité antimicrobienne de cinq plantes de la pharmacopée traditionnelle de l’Adamaoua de l’Adamaoua (cameroun). Cameroun J. Exp. Biol. 2011; 7(1):22-27.
20. Bokia N’Dongou Kolongo CBD. Évaluation des effets anti-inflammatoire et antalgique de l’aqueux des feuilles de Chromolaena odorata (L) King and Robinson (Asteraceae). Mémoire de Master de l’Université Marien Ngouabi, Brazzaville; 2016, p 48.
21. Elion Itou R. D. G, Etou Ossibi A. W, Epa C., Neondé Ntandou G. F, Bokia C. B, Ouamba J. M, and Abena A. A. Anti-inflammatory and analgesic effects of leaves of Chromolaena odorata L. (King and Robinson). African Journal of Pharmacy and Pharmacology. 2017; 11(17):217-223
22. Elion Itou R. D. G, Etou Ossibi A. W, Neondé Ntandou FQ, Abena AA. Antidiarrheal effect of stem bark of Ceiba pentandra Gaertn (Bombaceae) in rats. International Journal of Pharmaceutical Sciences and Research. 2018b, 9(5):2058-2061
23. Koutcheu Mabeku LB, Penlap Beng V, Kouam J, Ngadjui BT, Fomum ZT, Etoh FA. Evaluation of antidiarrheal activity of the stem bark of Cylindicus gabunensis (Mimosaceae). African Journal of Biotechnology. 2006; 5 (1):106-1066
24. GagnéT. S & Phillips S. F. . Ricinoleic acid; current view of its properties of Pentaclethra macrophylla leaf extracts. Journal of Applied Pharmacceutical Sciences. 2015; 27:41-93
25. Elion Itou R. D. G, Etou Ossibi A.W, Abena A. A. Antidiarrheal activity of methanolic extract of Oxalis barrelieri (Mimosaceae). African Journal of Pharmacy and Pharmacology. 2017; 11:77-82
26. Kouam J, Neondé Ntandou G, Elion Itou R. D. G, Mayela Nkouka S. H, Gouollaly Tsiba, Kassé D, Etou Ossibi A.W, Nsondé Ntandou G. F, Elion Itou R. D. G, Mayela Nkouka S. H, J., Gouollaly Tsiba, Kiéssé D, Etou Ossibi A.W and Abena A. A. Antiulcerogenic and Antioxidative effects of aqueous extract of Chromolaena odorata L. (King and Robinson). World Journal of Pharmaceutical and Life Sciences. 2018a; 4(2):47-52
27. Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 1983; 16(2):109-10.
28. Tagne Fokam MA, Kamgang R, Noubia PA, Oyono Essame J.L. Activity of Oxalis barrellieri aqueous extract on rat secretory diarrhea and intestine transit. Journal of Applied Pharmaceutical Science. 2015; 5(01):058-062
29. Coupier I. M. Opoid action of the intestine: the importance of the intestinal mucosa. Life Sci. 1987; 41:917–25.
30. Akah P. A, Aguala C. N, & Agu R. U. Studies on the antidiarrheal properties of Pentaclethra macrophylla leaf extracts. Phytother Res. 1999; 13:292–95.
31. Ngo Teke Gerald, Kuitse Jules-Roger, Kuéte Victor, Teponno Rémy Bertrand, Azeafack Léon Tapondjou, Vilarem Gerard. Antidiarrheal activity of extracts and compound from Tribilipedium madagascariense stem bark. Indian J Pharmacol. 2010; 42(3):157-163.
32. Khalilur Rahman Md, MD Ashraf Uddin Chowdhury, Mohammed Tawfiqul Islam, MD Anisuzzaman Chowdhury, Muhammad Erfan Uddin, and Chandra Datta Sumi (2015). Evaluation of Antidiarrheal Activity of Methanolic Extract of Maranta arundinacea Linn. Leaves. Advances in Pharmacological Sciences. 2015; 6 p.
33. Bahi BH, Kale RS. Antidiarrheal activity of ethanolic extract of Manihot esculenta Crantz leaves in Wistar rats. J Ayurveda Integr Med. 2015; 6(1):35–40