RJMP Research Journal of Medicinal Plants

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# *In-vivo* and *ex-vivo* Evaluation of Constipating and Spasmolytic Activity of *Dialium guineense* Wild Fruit Pulp

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## ABSTRACT

**Background and Objective:** *Dialium guineense* wild fruit pulp is highly appreciated by all socio-economic strata of Togo, but unfortunately, a significant portion of consumers complain of constipation after taking this pulp. This study aims to evaluate the constipating effects of the fruit pulp of *Dialium guineense*. **Material and Methods:** The effects of *Dialium guineense* fruit pulp (250, 500 and 1000 mg $kg^{-1}$) on the frequency of defecation, the quantity and the water content of the daily stools emitted by Wistar rats were studied. The effects of this pulp (1, 2, 4 mg $mL^{-1}$) on the motility of isolated rabbit ileum were also studied. **Results:** The fruit pulp of *D. guineense* at 250, 500 and 1000 mg $kg^{-1}$ have induced a significant decrease in the daily fecal number, fecal weight and water content. *D. guineense* pulp has also induced a significant ($p<0.001$) reduction in fecal number, fecal weight and water content after 7 days of administration. The fruit pulp of *D. guineense* at 1, 2 and 4 mg $mL^{-1}$ have induced a significant spasmolytic effect on the rabbit intestine at 8.88, 18.36 and 51.52%, respectively. **Conclusion:** The fruit pulp of *Dialium guineense* at different concentrations has constipating and spasmolytic properties and thus confirm the traditional claim of the constipating effect of the fruit pulp of *Dialium guineense*.

## KEYWORDS

*Dialium guineense*, fruit pulp, constipation, spasmolytic activity, rabbit ileum, Wistar rat



## INTRODUCTION

In Togo, the fruit pulp of *Dialium guineense* Wild (*D. guineense*) is highly appreciated by all socio-economic strata for its slightly sweet taste and its lower cost. According to studies conducted by Asoiro *et al.*[1], the pulp of these fruits is full of huge amounts of nutrients such as carbohydrates, lipids, proteins, minerals (sodium, magnesium, phosphorus, calcium and iron), as well as vitamins, such as vitamin C, thiamin, riboflavin and niacin. But unfortunately, a significant portion of consumers complain of constipation after taking this pulp. According to the literature, few scientific data mention this constipating effect of *Dialium guineense* fruits.

ISSN: 2151-7924 (Online)
ISSN: 1819-3455 (Print)
https://doi.org/10.3923/rjmp.2023.49.55

Received: 31 Mar. 2023
Accepted: 29 Aug. 2023
Published: 02 Sep. 2023

In Togo, the fruit pulp of *Dialium guineense* wild (*D. guineense*) is highly appreciated by all socio-economic strata for its slightly sweet taste and its lower cost. According to studies conducted by Asoiro *et al.*[1], the pulp of these fruits is full of huge amounts of nutrients such as carbohydrates, lipids, proteins, minerals (sodium, magnesium, phosphorus, calcium and iron), as well as vitamins, such as vitamin C, thiamin, riboflavin and niacin. But unfortunately, a significant portion of consumers complain of constipation after taking this pulp. According to the literature, few scientific data mention this constipating effect of *Dialium guineense* fruits.

Constipation results in a decrease in the frequency of bowel movements to less than three times a week in humans[2,3], a feeling of discomfort in the stomach, cramps, associated or not associated with bloating[3] with hard stools, little abundant and their expulsion seems incomplete[4-6]. It can manifest as the accumulation of a large number of feces in the rectal bulb or an excessive fecal load in the colon, rectum, or both[7]. This pathology creates a situation of discomfort for affected patients[6]. According to a meta-analysis carried out by Nascimbeni *et al.*[8] in 2002, constipation may be directly or indirectly involved in the development of colon cancer in subjects suffering from chronic constipation[7,9].

The objective of this work was, therefore, to evaluate the effects of this pulp on the frequency of defecation, the quantity and the water content of the stools emitted daily in Wistar rats and its effects on the intestinal motility of the rabbit, in ex-alive.

## MATERIALS AND METHODS

**Study area:** *Dialium guineense* pulp (10 g) was collected in Lomé, Togo market in July, 2020. It was shade-dried and ground to a powder. The study was conducted in the Toxicology, Department of the Faculty of Health Sciences, University of Lome, Togo from July to December, 2021.

**Animal material:** Twenty-five male Wistar rats of 200-250 g were used. Rabbits of 1.5 and 2 kg were also used. Animals were obtained from the animal facility of the Training Center on Medicinal Plants (CERFOPLAM) of the Department of Physiology and Pharmacology of the Faculty of Sciences of the University of Lomé. Animal care and handling were consistent with accepted guidelines[10,11]. They were kept in wooden cages with iron grids at a temperature of 23±3°C and on a light/dark cycle of 12 hrs with free access to food and water (except on days of experience). Good hygiene was maintained by constant cleaning and disposal of stool. These animals were acclimatized to laboratory conditions for one week before each experiment.

**Plant material:** The plant material is the fruit pulp of *D. guineense*. These fruits were bought at the "Agoe Assiyeye" market, a local market of Lomé. Loperamide, a synthetic opioid agonist, has been used as a reference drug for the induction of constipation.

**Preparation of powder from the fruit pulp of *D. guineense*:** The fruits were treated according to the method used by Asoiro *et al.*[1]. These fruits have been manually cleaned to remove waste as well as immature fruits. The healthy fruits obtained were peeled by hand to free them from their pods. They were then scraped, still manually, to separate the pulp from the seeds. The pulp thus obtained was dried under air conditioning and then reduced to powder until it was completely dehydrated. This powder is stored at -20°C until it is used.

**Evaluation of the constipating activity of the fruit pulp of *D. guineense*:** The method of Vanjari *et al.*[10] was used. Twenty-five Wistar rats weighing 200 to 250 g were fasted for 12 hrs with free access to water and were divided into five groups of five rats, in metabolic cages. Each group received daily for 7 days,

a specific treatment. The first group (group 1) received distilled water for all seven days (negative control). The second group (group 2) received 1 mg $kg^{-1}$ of Loperamide dissolved in distilled water (positive control), the 3rd, 4th and 5th groups received 250, respectively, 500 and 1000 mg $kg^{-1}$ of *D. guineense* fruit pulp dissolved in distilled water.

The stools passed by each group were collected every day at 9:00 a.m. from day zero. These stools were counted and then weighed to record their fresh weight. They were then dried under air conditioning until their complete dehydration (constant weight over time) in order to have the dry weight. The water content was then calculated using the following formula[10]:

$$\text{Water content} = \frac{\text{Fresh mass - dry mass}}{\text{Fresh mass}} \times 100$$

**Evaluation of the effects of *D. guineense* fruit pulp on rabbit gut motility in *ex-vivo*:** The Komla *et al.*[11] methods were used. Two rabbits weighing 1.5 and 2 kg were fasted for 24 hrs and then sacrificed. Once asleep, a midline abdominal incision was made to free the intestines from which the ileum was removed and immediately placed in oxygenated Ringer's solution. A portion of the ileum was then cut into several 2-4 cm fragments for assembly and experimentation in the isolated organ tank, thermostated at 37°C. One end of the isolated intestine fragment was fixed to the hook of the support rod and the other was fixed to the isometric sensor which itself is connected to a screen via an amplifier which makes it possible to visualize the spontaneous and regular contractions of the intestine isolated. The physiological solution was renewed every 15 min. After a stabilization period of 30 to 45 min, the basal tension of the intestinal fragment was recorded using the "LabChart 8.1.25 Reader" software and maintained at 3 g. Subsequently, a solution of *D. guineense* fruit pulp was added cumulatively to have final concentrations of 1, 2 and 4 mg $mL^{-1}$. The changes have been automatically saved. Animal care and handling were consistent with accepted guidelines. Ethical approval was obtained from the Committee on Institutional Ethics in Education and Research under issue (Ref. CNCB-CEER 2801/2010).

**Statistical analysis:** The results are expressed as the mean±standard error of the mean (SEM). Data were analyzed using SPSS version 25 software. All pooled data were statistically assessed and the significance of various treatments except those in the *in-vivo* test was calculated using One-way Analysis Variances (ANOVA) followed by Tukey's *post hoc* test were done. As $p<0.05$ was considered statistically significant.

## RESULTS

**Effects of *D. guineense* fruit pulp on the daily fecal number, weight and water content in rats:** The fruit pulp of *D. guineense* at 250, 500 and 1000 mg $kg^{-1}$ have induced a significant decrease in the daily fecal number of 14.81, 29.39 and 35.92% respectively (Fig. 1a). Whereas the reduction obtained with loperamide was 32.16%. Mean fresh feces mass decreased significantly with all three doses. The decrease is respectively 8, 20.56 and 29.30%. The reduction obtained with loperamide is 9.73% (Fig. 1b). The water content also decreased after the administration of *D guineense*, this decrease was 18.68% with the dose of 250 mg $kg^{-1}$, 38.35% with the dose of 500 and 63.68% with the dose of 1000 mg $kg^{-1}$. As for loperamide, it caused a decrease of 18.47% (Fig. 1c).

**Effects of *D. guineense* fruit pulp on the number, weight and water content of feces during the 7 days of treatment:** *Dialium guineense* pulp induced a significant ($p<0.001$) reduction in fecal number (Fig. 2a), fecal weight (Fig. 2b) and water content (Fig. 2c) after 7 days of treatment. With *D. guineense* at 1000 mg $kg^{-1}$, the reduction in the number of feces was 32.17% ($p<0.001$) whereas with loparamide the reduction was 33.91% ($p<0.001$). The reduction in the weight of feces was 27.78% with *D. guineense* at 1000 mg $kg^{-1}$ ($p<0.001$) whereas it was 5.56% with loperamide ($p<0.05$). The decrease in the water content was 69.05% with *D. guineense* at 1000 mg $kg^{-1}$ ($p<0.001$) while with loperamide it was 14.63% ($p<0.05$).

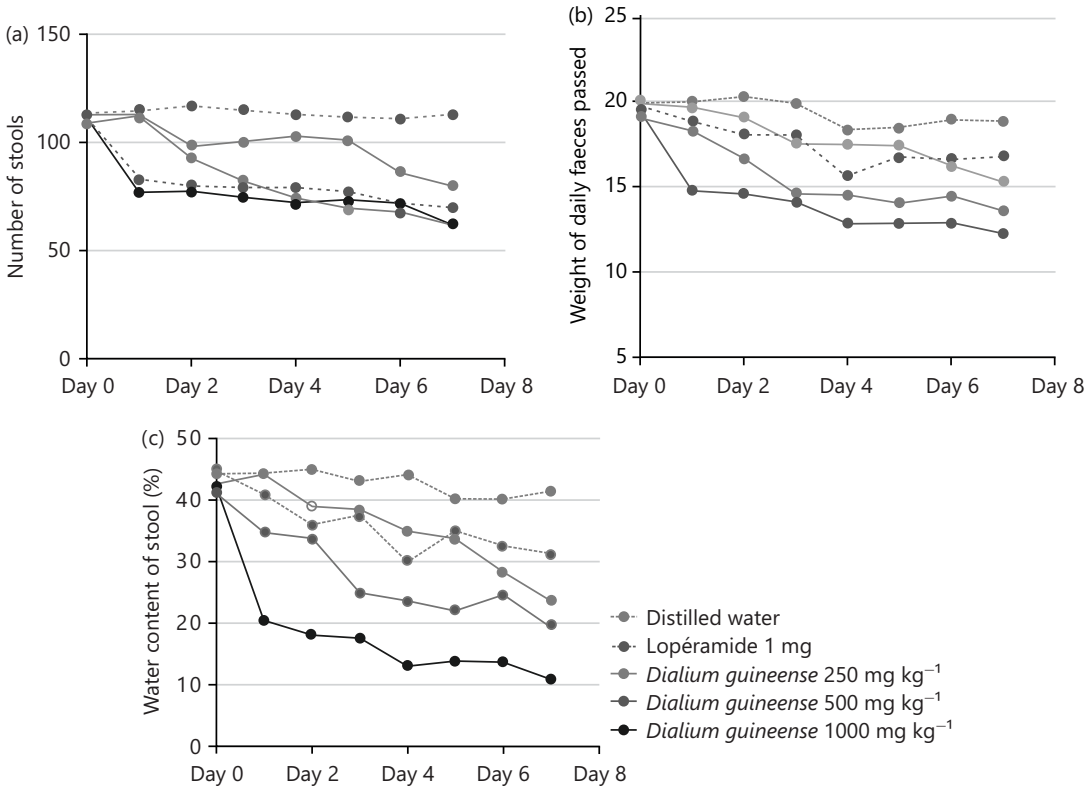


Fig. 1(a-c): Daily evolution of the (a) Number, (b) Weight in g and (c) Water content of feces emitted by the different groups

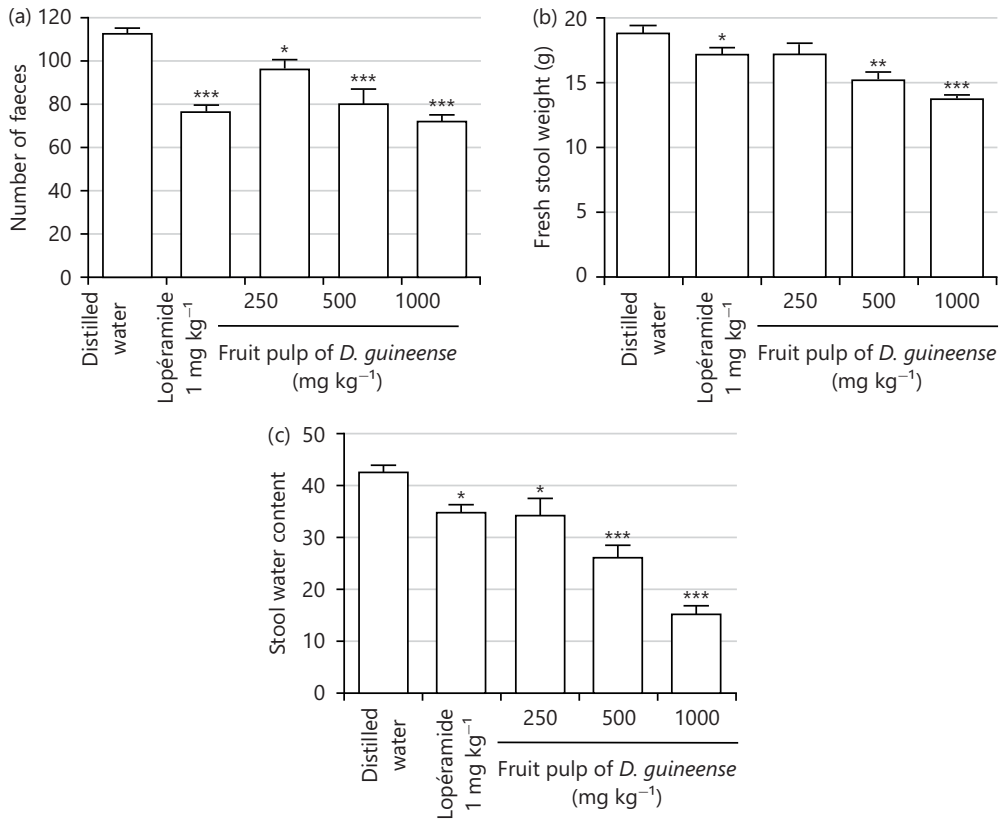


Fig. 2(a-c): Effects of *D. guineense* fruit pulp on the (a) Number, (b) Weight and (c) Water content of feces for 7 days of treatment

Data is expressed on average±SEM with n = 5, degree of significance *p<0.05, **p<0.01 and ***p<0.001 compared to the negative control

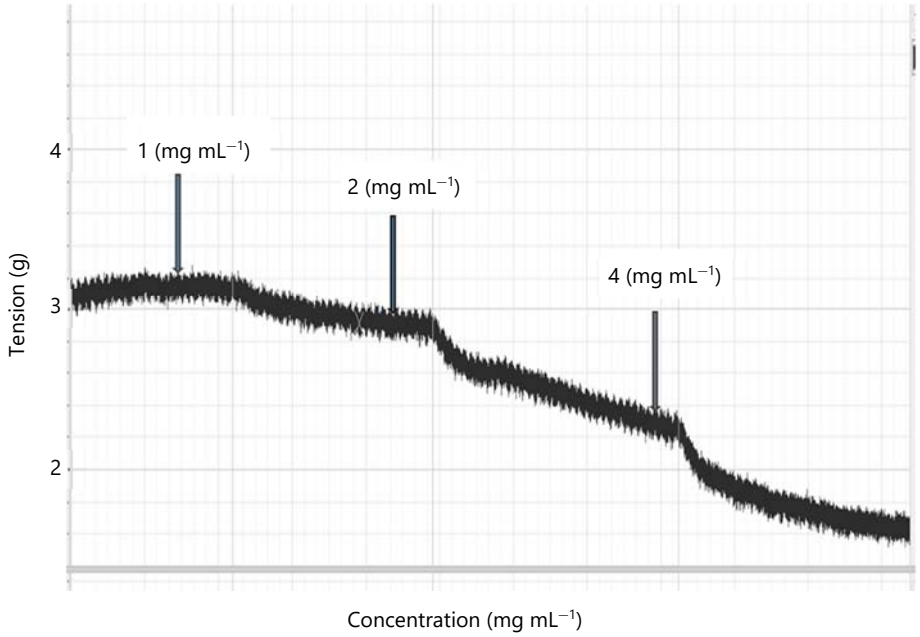


Fig. 3: Effects of the fruit pulp of *D. guineense* on the basal motility of the rabbit intestine in *ex-vivo*
Rabbits were fasted for 24 hrs and then sacrificed, a portion of the ileum was placed in isolated organ tank, thermos stated at 37°C, an intestine fragment was fixed to the isometric sensor, the basal tension of the intestinal fragment was recorded using the "LabChart 8 Reader" software and maintained at 3 g, subsequently, a solution of *D. guineense* fruit pulp was added cumulatively to have final concentrations of 1, 2 and 4 mg $mL^{-1}$

**Effect of *D. guineense* fruit pulp on basal gut motility in *ex-vivo*:** The fruit pulp of *D. guineense* at 1, 2 and 4 mg $mL^{-1}$ induced a significant spasmolytic effect on the rabbit intestine (Fig. 3). A decrease of 8.88, 18.36 and 51.52% were observed with the concentrations of 1, 2 and 4 mg $mL^{-1}$, respectively.

## DISCUSSION

The fruit pulp of *D. guineense* is highly appreciated by all age groups of the Togolese population. This pulp is consumed raw or transformed into syrup (but always fresh), for marketing or family consumption. During the ripening period of these fruits, their marketing generates various activities for farmers, retailers and processors. But unfortunately, the consumption of these fruits would lead to constipation. Several studies have focused on the antidiarrheal properties of *D. guineense*[12-14] but few studies have focused on its effect in causing constipation.

Constipation is defined by a decrease in the frequency of defecation associated or not with stool consistency[2]. Current results showed a significant reduction in the number of feces emitted by rats treated with *D. guineense* pulp. The results also showed a reduction in the quantity (weight) and water content of stools passed by rats treated with *D. guineense*. These observed reductions confirmed the induction of constipation[15,16], in rats treated with *D. guineense* fruit pulp.

Regarding the effects of these fruits on rabbit intestinal motility, in *ex-vivo*, the results revealed a dose-dependent inhibition of baseline intestinal motility and these effects are cumulative. This spasmolytic activity confirmed the induction of constipation by the pulp of *D guineense*. The spasmolytic activity explains the decrease in the frequency of defecation in rats treated with the pulp of *D. guineense*.

*Dialium guineense* fruit pulp at doses of 500 and 1000 mg $kg^{-1}$, significantly reduced ($p<0.001$) the number of feces passed, as did loperamide at 1 mg $kg^{-1}$. However, its effects on stool weight and water content are very significant ($p<0.001$) compared to loperamide ($p<0.05$). This leads us to say that the fruit

pulp of *D. guineense* could induce constipation by a spasmolytic effect just like loperamide and by an acceleration of the absorption or inhibition of the secretion of water and electrolytes in the lumen of the digestive tract, which is manifested by a reduction in the water content of feces. Current results supported the traditional use of *D. guineense* in the management of diarrhea[17].

However, the evaluation of the mechanism of action will help to monitor its use. Current results confirmed the side effects mentioned by *D. guineense* consumers who often say that the pulp gives constipation. *Dialium guineense* fruit pulp could therefore have antidiarrheal effects and it should therefore be discouraged for people suffering from chronic constipation.

## CONCLUSION

The fruit pulp of *D. guineense* significantly reduced the frequency of defecation, the number of feces passed as well as the water content of these feces. This activity would be due to the spasmolytic properties possessed by this pulp. Although further studies are warranted using different models, the results of our study confirmed the traditional claim of constipating activity of *D. guineense* fruit pulp.

## SIGNIFICANCE STATEMENT

The purpose of this study is to confirm or invalidate the claims that dialium has the power to induce constipation. The results have shown the effect of *D. guineense* on intestinal transit and confirmed these suspicions. Consequently, it would be good to advise against this pulp for patients suffering from chronic constipation. *Dialium guineense* may be beneficial for the treatment of diarrhea. This study thus allows researchers to explore the antidiarrheal properties of *D. guineense*.

## ACKNOWLEDGMENTS

We thank the TWAS (The World Academy of Science) for its free funding (Grant No. 19-242 RG/BIO/AF/AC_G/) during the research work and the Pierre Fabre Foundation (France) for its support to the Department of Pharmacy of the University of Lome, Togo.

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