**In vitro antispasmodic effects of Mallotus oppositifolium leaves extracts**

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**Abstract**

*Mallotus oppositifolium* is a plant traditionally used in many African countries to treat diarrhea and other gastrointestinal tract disorders. This study was undertaken to evaluate *in vitro* antispasmodic effects of decoction, hexane and methanolic leaves extracts of this plant. Antispasmodic activities of *Mallotus oppositifolium* leaves decoction, hexane and methanolic extracts were assessed on spontaneous contractions, on acetylcholine or potassium chloride (KCl) induced contractions of isolated rat duodenum strips. *Mallotus oppositifolium* decoction, hexane or methanolic extracts increasing cumulative concentrations relaxed duodenum strips and at 1 mg/mL, they respectively decreased contraction amplitudes by 77.84 ± 2.25, 55.81 ± 2.14, and 51.67 ± 0.95% compared to the initial values. Decoction, hexane, and methanolic extracts decreased contraction tensions respectively from 3.00 to 2.5 gF, 3.40 to 2.80 gF and from 3.10 to 2.50 gF. At 0.25 mg/mL, they significantly (p <0.05) decreased contraction tension. Emax were 186.89 ± 34.05, 116.30 ± 10.92 and 91.57 ± 4.70% respectively with decoction, methanolic, and hexane extracts with EC$_{50}$ of 0.610 ± 0.184; 0.146 ± 0.011 and 0.237 ± 0.105 mg/mL (p <0.05). Acetylcholine increased contraction amplitudes. Cumulative administration of *Mallotus oppositifolium* decoction at 0.05 to 2.00 mg/mL significantly (p <0.05) decreased tension, from 4.00 to 2.60 g.F. At these concentrations, tension variation was concentration-dependent, it increased from 13.39 ± 1.72 to 77.59 ± 1.10%. EC$_{50}$ and Emax were respectively 1.311 ± 0.340 mg/mL and

**Keywords:** mallotus oppositifolium; acetylcholine; potassium chloride; antispasmodic effects; gastrointestinal tract disorders.
Mallotus oppositifolium extract leaves were collected from the in a rotary evaporator at 40-45°C to obtain 28.6 g (5.72%) of solution of brown and homogeneous color was concentrated filtrate obtained, a semi-solid cotton and subsequently using Whatman No. 1 filter paper. The electric blender and kept in polyethylene bags ready for extrac fore being air-dried for 2 weeks at room temperature (22-26°C).

The leaves were thoroughly rinsed twice in running tap water and then in sterile water be determined according to the dilution formula:

\[
\text{IC} \times \text{IV} = \text{FC} \times \text{FV} \leftrightarrow \text{FV} = \left( \frac{\text{IC} \times \text{IV}}{\text{FC}} \right)
\]

- IC the initial concentration of the substance to be administered;
- IV the initial volume of the substance to be administered;
- FC the final concentration of the substance in Tyrode's liq uid;
- FV the final volume of Tyrode's physiological solution.

The present study was undertaken to evaluate effects of decoction, hexane and methanolic extracts of Mallotus oppositifolium leaves on duodenum strips spontaneous contractions as well as on acetylcholine or potassium chloride duodenum-induced contractions.

Materials and methods

Plant material and preparation of the extract and fractions

Mallotus oppositifolium leaves were collected from the Gouife-Bep village (Bafia-Cameroon) on October 2012. The species was identified and authenticated in the National Herbarium of Cameroon by comparison with a voucher specimen deposited under the number HNC 16619. The leaves were thoroughly rinsed twice in running tap water and then in sterile water be before being air-dried for 2 weeks at room temperature (22-26°C). The dried leaves were powdered into a fine texture using an electric blender and kept in polyethylene bags ready for extraction.

Mallotus oppositifolium leaves aqueous extract (decoction)

Mallotus oppositifolium leaves powder (500 g) was macerated in 5 L of distilled water for two hours. The macerate was boiled (15 min) in a water bath and then, first filtered through cotton and subsequently using Whatman No. 1 filter paper. The filtrate obtained, a semi-solid Mallotus oppositifolium extract solution of brown and homogeneous color was concentrated in a rotary evaporator at 40-45°C to obtain 28.6 g (5.72%) of decoction (DEMO).

Mallotus oppositifolium hexane and methanol extract

Mallotus oppositifolium leaves powder (500 g X 2) was macerated respectively in 5 L of methanol or in 5 L of hexane to obtain methanol (MEMO) or hexane (HEMO) filtrates which were then concentrated to 200 millibars in rotary evaporators at 40°C for the methanol or at 45°C for the hexane filtrates, to respectively obtain 29.6 g (5.92%) of methanolic extract (MEMO) or 27.3 (5.46%) of hexane extract (HEMO).

Experimental animals

The experiments were carried out on Wistar albino rats of both sexes, reared at room temperature, with sufficient aeration, in animal house of the Institute for Medical Research and Study of Medicinal Plants (IMPM) and at the Faculty of Medicine and Biomedical Sciences (FMSB) of the University of Yaounde I. Animals ad libitum had access to tap water supplemented with a few drops of vitamin B complex (5 drops per liter). Their diet was made up of a mixture of corn flour (60%), wheat (10%), fish (12%), soybean (15%) and 3% red oil [11].

Experimentation

Effects of Mallotus oppositifolium extracts on rat isolated duodenum fragments spontaneous contractions

Reagents and experimental physiological solution

Effects of M. oppositifolium aqueous, hexane or methanol extracts were evaluated on duodenum motility with the aid of an organ bath (20 mL) containing Tyrode’s physiological solution. Its composition in mM (NaCl 136.90; Glucose 5.55; NaHCO3 11.90; CaCl2 1.87; KCl 2.68; MgCl2 1.05, and NaH2PO4 0.42) is suitable to maintain alive duodenum strips [12]. Experiments were carried out on normal and on Acetylcholine or Potassium Chloride-precontracted duodenum strips. Stock solutions were prepared by dissolving 3.05 mg of acetylcholine chloride (SIGMA Chemical Co., ST Louis, USA) in 134 mL of distilled water. (80 μM) or 746 mg of Potassium Chloride salt in 5 mL of distilled water (50 mM).

Administration of any substance in the bath was preceded by a certain volume of Tyrode’s solution which thereafter was adjusted to 20 mL with the corresponding volume of the said substance. The final concentration of drugs in the bath was determined according to the dilution formula:

\[
\text{IC} \times \text{IV} = \text{FC} \times \text{FV} \leftrightarrow \text{FV} = \left( \frac{\text{IC} \times \text{IV}}{\text{FC}} \right)
\]
In our study, from stock extract solutions of a concentration of 100 mg/mL, final extract concentrations of 0.0125, 0.05, 0.25, 0.50, 1.00, 2.00, and 4.00 mg/mL were tested by, respectively, adding into the organ bath 0.0062, 0.125, 0.025, 0.05, 0.10, and 0.2 mL of the stock solutions.

**Experimental design**

Rats were fasted for 24 h before the experiments with free access to water. Then they were sacrificed by cervical dislocation, the abdomens were cut open and the duodenal portions were isolated. Preparations of about 2 cm long were mounted in 20 mL tissue baths containing Tyrode’s solution maintained at 37°C, constantly aerated with carbogen (a mixture of 5% carbon dioxide in oxygen). Tension of about 1.0 gF of preload was applied to tissues and subsequently kept undisturbed for one hour for stabilization.

The registration section was made up of a Student Lab software connected to the acquisition system (Biopac MP 35), equipped with an isometric transducer which transformed the mechanical contractile activity of the smooth muscles of the intestinal fragment into electric signals; visualized through a computer.

**Evaluation of different extracts of *Mallotus oppositifolium* on rats duodenum spontaneous contractions**

Spontaneous contractions of duodenum fragments in organ bath were observed during 60 min in order to obtain an approximately constant amplitude, frequency and tone (stabilization). During this stabilization process, Tyrode’s solution in the organ bath was replaced every 15 min after which a tension of 1 g F was applied to the organ.

The first series of experiments was carried out to evaluate effects of different extracts of *M. oppositifolium* (decocition, methanolic, hexane) in increasing concentrations (0.0125 to 2 mg/mL) on duodenum contractions. After stabilization, a certain volume of extract solution was added into the organ bath and its effects on spontaneous contractions were observed over a period of 20 min. The organ was then washed 3 to 4 times with fresh Tyrode’s solution and left for complete recovery for 60 min before being resubjected to a second series of the same extract concentrations as previously done. The effects on rat duodenum contractions were evaluated in at final concentrations of 0.0125, 0.05, 0.25, 0.50, 1.00, and 2.00 mg/mL. Distilled water was used as control. The concentration-response curve of the extract was obtained from a non-linear regression using GraphPad Prism software version 5.03. The extract concentration which caused half of the maximum effect (EC_{50}), as well as extract concentration which caused the maximum effect (Emax) were determined [13,14].

The effects of *M. oppositifolium* extracts were also evaluated on the parameters of contractions, such as amplitude, and basic tension.

**Evaluation of the effects of *Mallotus oppositifolium* extracts on variations of amplitude of spontaneous contractions**

Contraction amplitudes were recorded before (ampl. T0) and after application of extracts (ampl. Ti). After administration of the extract, amplitude of contractions was recorded every 2 min for 20 min and the contraction amplitude variation (in percentage) was calculated as follow [13,14]:

\[
\text{Contraction Amplitude variation (\%)} = 100 \times \left( \frac{\text{ampl. Ti} - \text{ampl. T0}}{\text{ampl. T0}} \right)
\]

ampl.Ti = amplitude at a specific time after administration of the extract
ampl.T0 = mean amplitude before administration of the extract

**Evaluation of the effects of *Mallotus oppositifolium* extracts on spontaneous contraction basic tension**

The initial tension mean (T_i) is the basic tension recorded before extract administration. Tension variation (in percentage) was expressed according to the initial tension mean as follows [13,14]:

\[
\text{Tension Variation (\%)} = 100 \times \left( \frac{T_i - T_0}{T_m} \right)
\]

T0 = initial tension mean (average of minimum tensions before extract administration)
Ti = minimum tenion of the duodenum segments recorded for 2 min
Tm = minimum tension of the duodenum segments during the 20 min of experimentation

After extract administration, basic contractions tensions were recorded every two minutes during the 20 min of experimentation.

Investigation among local population reveals that decoction of the plant was orally taken in the management of diarrheal syndromes. Moreover, preliminary results did not show great difference between the effects of the different extracts. In addition, the methanol and hexane used for the said extracts could be harmful to the body. Hence, we choose the decoction for further evaluation of their antidiarrheal properties of this plant.

**Evaluation of the effects of *Mallotus oppositifolium* decoction on acetylcholine or potassium chloride-induced duodenum contraction**

Effects of *Mallotus oppositifolium* aqueous extract were evaluated on acetylcholine or potassium chloride induced duodenum contraction. Each duodenum segment was mounted in the organ bath containing 15 mL of Tyrode’s solution maintained at 37°C and continuously ventilated with a mixture of 95% oxygen and 5% carbon dioxide (carbogen). Duodenum segments were left in calibration and stabilization for 60 min before any drug administration. Acetylcholine (80 µM) was then injected into the organ bath to induce muscle contraction. Once the tension of the contraction was stable, every 5 min, *Mallotus oppositifo- lium* aqueous extract was added to the incubation medium at cumulative concentrations ranging from 0.0125 to 2.00 mg/mL. A concentration-response curve of extract effects was obtained; the median effective concentration (EC_{50}) and maximum muscle relaxant effect (Emax) were determined.

Under the same experimental conditions, *Mallotus opposi- folium* aqueous extract effects were evaluated on potassium chloride (50 mM) induced contraction. After calibration and stabilization of duodenum segments, KCl solution was injected into the organ bath (at a volume corresponding to a final con-
centration of 50 mM). Once the contraction was stable, every 5 min, *Mallotus oppositifolium* aqueous extract was added to the incubation medium at cumulative concentrations ranging from 0.0125 to 2.00 mg/mL. From the concentration-response curve, the median effective concentration (EC$_{50}$) and maximum muscle relaxant effect (Emax) were determined [12].

**Statistical analysis and presentation of results**

Results were expressed as Mean ± Standard Error of the Mean (SEM) of several reproduced tests. Duodenum motility, changes in amplitude and tension of contractions were assessed and expressed in percentage compared to their initial values. Graph Pad Prism software version 5.03 (GraphPad Software, San Diego, California, USA) was used to perform statistical analysis. Statistical differences between control and treated groups were tested by a one-way analysis of variance (ANOVA), followed by Tukey’s multiple comparison test. Values of p <0.05 were considered statistically significant.

**Results**

**Effects of *Mallotus oppositifolium* extracts on rat duodenum spontaneous activity**

Spontaneous contractions of the rat duodenum (Figure 1) are oscillations of more or less variable tension and amplitude, characteristic of intestine peristalsis.

Administration of increasing cumulative concentrations (0.0125, 0.05, 0.25, 0.50 and 1.00 mg/mL) of *M. oppositifolium* aqueous, hexane and methanol extracts in the incubation bath produced relaxation of duodenum segments. Aqueous, methanol, and hexane extracts at 0.0125 mg/mL decreased contraction amplitudes respectively by 12.71 ± 1.05 (Figures 2a), 1.09 ± 0.04 (Figures 2b), and 13.07 ± 1.61% (Figures 2c). At 1 mg/mL, these extracts, decreased contraction amplitudes respectively by 77.84 ± 2.25% (Figure 2a), 55.81 ± 2.14% (Figure 2b) and 51.67 ± 0.95% (Figure 2c) compared to the initial values. Cumulative extract concentrations (0.0125 to 1 mg/mL) decreased in a dose-dependent way, tension of duodenum contractions. Treated with *M. oppositifolium* aqueous, hexane, and methanolic extracts, tensions respectively went from 3.00 to 2.5 gF, 3.40 to 2.80 gF and from 3.10 to 2.50 gF (Figure 3a). *M. oppositifolium* aqueous, hexane and methanolic extracts at 0.25 mg/mL, significantly (p <0.05) decreased contraction tension and the decrease percentages were respectively 82.65 ± 5.40, 62.00 ± 2.06, and 52.06 ± 1.50%. At 1 mg/mL, these percentages were respectively 83.87 ± 3.15, 62.53 ± 5.85, and 104.90 ± 4.04% (p <0.05). Maximum muscle relaxant effects (Emax) observed were 186.89 ± 34.05, 116.30 ± 10.92 and 91.57 ± 4.70% respectively with aqueous, methanolic, and hexane extracts. Extract concentrations causing 50% muscle relaxation (Figure 3b) or median effective concentrations (EC$_{50}$) were respectively 0.610± 0.184; 0.146 ± 0.011 and 0.237 ± 0.105 mg/mL (p <0.05) for the three extracts. The relaxing effect of *M. oppositifolium* extracts was stable after 2-3 recordings indicating that it was not a toxic effect.

**Effects of *Mallotus oppositifolium* decoction on acetylcholine-induced duodenum contractions**

Administration of a single dose of acetylcholine (ACh), 80 µM in the incubation bath caused rapid phasic contraction followed by a slowly decreasing tension. ACh at this concentration, induced an increase of contraction amplitudes which further gradually decreased. Tension increased, due to ACh from 4.20 to 5.00 gF (Figure 4a) corresponding to an increase of 25.68 ±
1.50%, compared to 8.25 ± 2.09% in the control treated with distilled water (Figure 4b). Cumulative administration of *M. oppositifolium* aqueous extract from 0.05 to 2.00 mg/mL caused a significant (p <0.05) decrease of tension, from 4.00 to 2.60 gF. At these extract concentrations, tension variation of spontaneous contractions was concentration-dependent and it increased from 13.39 ± 1.72 to 77.59 ± 1.10%. Median effective concentration (EC$_{50}$) and the maximum muscle relaxant effect (Emax) of the extract, determined from the analysis of the concentration–response curves were respectively 1.311 ± 0.340 mg/mL and 99.29 ± 24.80% (Figure 5).

**Figure 4:** Contractions of the duodenum induced by acetylcholine alone (a) and in the presence of cumulative doses of *M. oppositifolium* aqueous extract (b).

**Figure 5:** Contraction tension of rat duodenum induced by Ach and in the presence of the different cumulative *M. oppositifolium* aqueous extract doses. n=5, **p <0.05, ***p <0.01 significant differences compared to control (Distilled water).

**Figure 6:** Contraction tension of rat duodenum induced by Ach and in the presence of the different cumulative *M. oppositifolium* aqueous extract doses. n=5, * p <0.05, *** p <0.01 significant differences compared to control (Distilled water).

**Discussion**

*Mallotus oppositifolium* (Euphorbiaceae) has been used in Cameroon and other equatorial and tropical countries of Africa to treat many diseases. In those Regions, different parts of the plant are used in the management of affections such as helminthiasis, gastro-intestinal disorder like dysentery and acute diarrhea [6]. The leaves have several properties against diabetes, convulsions and epilepsy [7]. Anti-inflammatory [8], and antioxidant effects [9,10] of the plant have been carried out. The present study was undertaken to evaluated effects of decoction, hexane and methanolic *Mallotus oppositifolium* leaves extracts on duodenum strips spontaneous contractions as well as on acetylcholine or potassium chloride duodenum-induced contractions.

The duodenum is the initial portion of the small intestine and is where absorption actually begins. The duodenal wall has two regions of muscle tissue, the muscularis externa and the muscularis mucosa. The muscularis externa consists of an outer longitudinal layer and an inner circular layer and is the major effector of mixing and propulsion. The myenteric plexus of Auerbach lies between these two layers and with the Meissner plexus they constitute the enteric nervous system [15]. Uncontrolled contractions of these muscles can lead to diarrheal diseases, a serious major public health problem in developing countries [16]. Diarrhea is an intestinal disorders and/or infec-
Contractions of intestine smooth muscles are initiated by phosphorylation of the 20 kDa regulatory subunit of the myosin light chains (MLC20) by Ca\(^{2+}\)/calmodulin-dependent myosin light chain kinase or Ca\(^{2+}\)-independent kinases [22]. An increasing level of cytoplasmic Ca\(^{2+}\) ([Ca\(^{2+}\)]\(i\)) is the main physiological event that activates myosin light chain kinase. Phosphorylation of MLC20 facilitates myosin binding to actin, initiating cross-bridge cycling and force development. Neurotransmitters such as Acetylcholine (ACh) and potassium salts such as Potassium Chloride (KCl) are potential muscle contraction stimulators, which can accelerate intestinal transit and thus, causing diarrheal syndromes. Aqueous, hexane and methanol extracts of *M. oppositifolium* decreased amplitude and tension of rat duodenal spontaneous contractions. Administration of acetylcholine (ACh) in this organ bath caused a rapid phasic contraction followed by a tonic contraction which decreased slowly. ACh is released by the parasympathetic nervous system and is involved in the physiological regulation of intestinal transit [23]. Acetylcholine induces intestinal smooth muscle contractions through a double mechanism coupled to muscarinic receptors: one of the mechanisms is the non-selective activation of cation channels leading to membrane depolarization and calcium influx via voltage-gated calcium channels [24]. The other mechanism is the release of intracellular calcium from sarcoplasmic reticulum. The ability of *M. oppositifolium* extract to inhibit spontaneous or acetylcholine-induced contractions, indicates that the plant has anti-diarrheal effects through its myorelaxant activity [25]. Administration of Potassium Chloride in the incubation medium produced a sustained rapid phasic contraction followed by a slow tonic contraction whose tone was concentration-dependent inhibited by *Mallotus oppositifolium* decoction. A high extracellular concentration of K\(^+\) stimulates the opening of voltage-gated calcium channels, thus inducing the extracellular calcium influx leading to contractions [26, 27]. The ability of *M. oppositifolium* extract to inhibit normal spontaneous duodenal contractions and those induced by KCl shows that the plant would have antidiarrheal effects through its muscle relaxant activity on spontaneous duodenal contractions.

Qualitative and quantitative phytochemical studies revealed the presence of bioactive metabolites such as tannins, alkaloids, saponins, flavonoids, anthraquinones, steroids and/or terpenoids whose anti-diarrheal effects have been demonstrated [28,29]. Flavonoids, and tannins are generally responsible for myorelaxant properties of medicinal plants [29]. Further more, flavonoids showed their ability to inhibit contractions induced by spasmsogens as well as to reduce the amplitudes of intestine contractions through inhibition of acetylcholine secretion [30].

**Conclusion**

Based on the results, it can be concluded that the *Mallotus oppositifolium* both leaves decoction and hexane or methanolic extracts have myorelaxant properties probably through inhibition of acetylcholine release or its activities. *Mallotus oppositifolium* therefore, constitutes a potential candidate for the development of traditional improved medicines for the management and control of gastrointestinal disorders, especially diarrhoea. Future work is needed to isolate and characterize pure compounds which sustain these pharmacological activities.

**Declarations**

**Conflicts of interest**: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**References**


