



**ASSESSMENT OF ANTISPASMODIC EFFECTS OF POLYPHENOL AND COMPLEX CATECHIC TANNIN EXTRACTS FROM SOME MEDICINAL PLANTS USED TO TREAT DIARRHOEA IN TRADITIONAL MEDICINE IN KINSHASA-DEMOCRATIC REPUBLIC OF CONGO**

**Cimanga Kanyanga R.<sup>a,c\*</sup>, Kimbaira L.<sup>b</sup>, Tona L.G.<sup>b</sup>, Kambu K.O.<sup>a</sup>, Vlietinck A.J.<sup>c</sup>, Pieters L.<sup>c</sup>**

<sup>a</sup>Department of Medicinal Chemistry and Pharmacognosy, Laboratory of Pharmacognosy and Phytochemistry, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O.Box 212. Kinshasa XI. Democratic Republic of Congo.

<sup>b</sup>Department of Pharmacology and Therapeutics, Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, University of Kinshasa, P.O.Box 212, Kinshasa XI, Democratic Republic of Congo.

<sup>c</sup>Department of Pharmaceutical Sciences, Natural Products & Food Research and Analysis (NatuRA), University of Antwerp, Universiteitsplein 1, B-2610, Antwerp, Belgium.

Article Received on  
02 Nov. 2018,

Revised on 23 Nov. 2018,  
Accepted on 13 Dec. 2018

DOI: 10.20959/wjpps20191-12908

**\*Corresponding Author**

**Dr. Cimanga Kanyanga R.**

Department of Medicinal  
Chemistry and  
Pharmacognosy, Laboratory  
of Pharmacognosy and  
Phytochemistry, Faculty of  
Pharmaceutical Sciences,  
University of Kinshasa,  
P.O.Box 212. Kinshasa XI.  
Democratic Republic of  
Congo.

**ABSTRACT**

Polyphenol and complex catechic tannin extracts from some medicinal plants used as antidiarrheal remedies in traditional medicine in Kinshasa-Democratic Republic of Congo were submitted to the evaluation of their capacity to inhibit contractions induced by acetylcholine (ACh) and depolarizing solution rich in KCl (DSR-KCl) on isolated guinea-pig ileum. Against ACh effects, results revealed that five polyphenol extracts including those from *Maprounea africana* and *Mangifera indica* stem bark, *Morinda morinodoides*, *Psidium guajava* and *Tithonia diversifolia* leaves, inhibited contractions induced by this agonist by 80% < % inhibition < 90%. Seven polyphenol extracts from *Alchornea cordifolia*, *Cajan cajan*, *Nauclea latifolia* and *Morinda lucida* leaves, *Bridelia ferruginea* and *Garcinia kola* stem bark, and *Harugana madagascariensis* root bark produced an inhibition of contractions induced by ACh between 70 and 80% on isolated guinea-pig ileum. Two polyphenol extracts from *Paropsia brazzeana* root bark

and *Pentaclethra macrophylla* stem bark produced less than 55% inhibition of ACh effects. Among tannins extracts, those from *B. ferruginea* and *M. africana* stem bark, *H. madagascariensis* root bark and *M. morindoides* leaves produced more than 70% inhibition induced by ACh on isolated organ and other complex catechic tannin extracts from *C. cajan*, *N. latifolia*, *M. lucida* leaves, *B. ferruginea* and *G. kola* stem bark, and *H. madagascariensis* reached 60% % inhibition < 70% of contractions induced this agonist. Regarding their effects on DSR-KCl, results indicated that polyphenol extracts from four medicinal plants including *M. morinodoides* and *P. guajava* leaves, *M. africana* and *M. indica* stem bark caused an inhibition of contractions induced by this agonist ranging from 80 to 89% while those from *A. cordifolia*, *C. cajan*, *M. lucida* leaves, *G. kola* and *H. madagascariensis* and *M. africana* stem bark produced 70% < inhibition < 80% of its effects. In conclusion, these tested phytochemical groups including polyphenols and complex catechic tannins were found able to inhibit significantly contractions induced by both agonists showing their antispasmodic effect and can be in part, considered as responsible for the antidiarrhoeal properties attributed to these medicinal plants by different practioners in traditional medicine in Democratic Republic of Congo.

**KEYWORDS:** Medicinal plants, polyphenols, tannins, antispasmodic activity.

## 1. INTRODUCION

In developing countries, morbidity and mortality due to diarrhoea and other gastrointestinal conditions are a big sanitary problem. In spite of enormous development of synthetic antidiarrhoeal drugs in the mark such as diphenoxylate, atropine sulfate and loperamide which are currently used to treat the disease, their usage, however, may cause some side effects such as intestine obstruction, constipation, vomit and nausea (Pham et al., 2017). It was estimated 10 million deaths per year manly children under 5 years in the world (Barakat et al., 2013). Thus, people are still relying on herbal remedies for control and treatment of diarrhea and found some reliefs (Agunu et al., 2005). In addition, the world health organization (WHO) highly appreciated medicinal practices for the treatment and precautionary mesure of diarrheal diseases and has encouraged the use and studies of herbal remedies for the prevention and treatment of diarrhoea since 1980s due to their affordability as well as abundance (Shoba et al., 2014, Kavitha and Indira, 2016).

Nowadays, many medicinal plant claimed by traditional healers in their daily practices to treat diarrhoea are now scientifically investigated for putative antispasmodic activity in

different experimental model and many of them are proved to possess this biological activity (Gilani et al., 2010; Consolini et al., 2011; Irshad et al., 2012; Barakat et al., 2013; Prasama et al., 2013; Ali et al., 2014; Asifa et al., 2017; Chattida et al., 2017, Silva et al., 2017). In some cases, active principles were isolated (el-Shafae, 1998 ; de Moura et al., 2002, Mendel et al., 2016).

All medicinal plant selected in the present study are well known to be used as aqueous decoction or macerate in traditional medicine by different practioners in traditional medicine in Kinshasa-Democratic Republic of Congo to treat diarrhoea. In previous study to explore further medicinal potential, these medicinal plant species were reported to exhibit antispasmodic, antiamoebic and antibacterial with different magnitudes and these biological can in part, justify and support their use for the treatment of the disease cited above (Tona et al., 1999, Cimanga et al., 2018a,b). In a recent study on flavonoid extracts from these medicinal plant, it was reported that this phytochemical group exhibited significant antispasmodic activity at different extents (Cimanga et al., 2018c) an can be in part considered as one of the active phytochemical group for antidiarrheal properties attributed to these medicinal plant species. The present study was undertaken to evaluate antispasmodic activity of other phytochemical groups including polyphenols and complex catechic tannin obtained from the used preparations using each selected respective vegetal organ such as leaves, stem and root barks.

## **2. MATERIALS AND METHODS**

### **2.1. Plant materials**

All plant parts were collected around city of university of Kinshasa in Kinshasa-Democratic Republic of Congo. All plants were authenticated at the National Institute of Studies and Research in Agronomy (NISRA). A voucher specimen of each medicinal plant where deposited in the herbarium of this institute. All plant materials were dried at room temperature and reduced to powder using an electronic blender.

### **2.2. Extraction of polyphenol and complex catechic tannin extracts**

For polyphenol extract, 100 g of each powdered plant material were mixed with 300 ml distilled water and heated on a hotplate for 15 minutes. After cooling and filtration on filter paper Whatman N°1, each filtrate was exhaustively extracted with *n*-butanol, ethylacetate and isoamylic alcohol. Each organic phase was evapored *in vacuum* yielding corresponding dried

extract. These extracts gave positive test with  $\text{FeCl}_3$  for the presence of polyphenolic compound. They were combined in one extract constitute a total polyphenol extract.

Complex catechic tannins were extracted by using 30 ml of each aqueous decoction of each plant material mixed with Stiasny reagent ( $\text{HCl} + \text{formol}$ ) to give abundant brun precipitate (Tresae and Evans, 1996; Harborne, 1998). This was filtrated on paper filter Whitman N°1 and wash with diethylether. Each precipitate was recuperated on pare filter and dried at  $50^\circ\text{C}$  to give corresponding dried complex catechic tannins.

### 2.3. Assessment of spasmolytic activity

Male guinea-pig sex (220-250 g body weight) were anesthetized and sacrificed by cervical displacement followed by exsanguination. The ileum was dissected out (2-3 cm long), plentifully washed with distilled water and suspended (3 cm of ileum) in an organ bath containing 50 ml of Tyrode's solution (mM:  $\text{KCl}$ : 2.2,  $\text{MgCl}_2$ : 0.11,  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ : 0.42,  $\text{CaCl}_2$ :1.8,  $\text{NaCl}$ :137,  $\text{NaHCO}_3$ :11, glucose:5.6) or depolarizing solution rich in  $\text{KCl}$  (mM:  $\text{NaCl}$ : 2.7,  $\text{KCl}$ :100,  $\text{NaHCO}_3$ :15,  $\text{CaCl}_2$ :1.25,  $\text{MgCl}_2$ :12.5, glucose:11) gassed with 95%  $\text{O}_2$  and 5%  $\text{CO}_2$  according the case.

The isolated tissue was allowed to equilibrate for 30 minutes under a resting tension of 0.5 g before exposure to drugs and tested samples. To evaluate antispasmodic activity, the tissue was first exposed to  $5 \cdot 10^{-7}$  M acetylcholine (ACh) or depolarizing solution rich in  $\text{KCl}$  (DSR  $\text{KCl}$ ) to have tree equivalent amplitudes of contractions and was after plentifully washed with Tyrode's solution to eliminate the presence of agonists in the organ bath. 5 mg of tested samples (polyphenols or tannins) were dissolved in 5 ml distilled water to have stocks solution of 1 mg/ml. After, 2 ml of agonists were removed in organ bath and replace by 1 ml of tested samples corresponding to 20  $\mu\text{g}/\text{ml}$  in organ bath and left in contact with isolated guinea-pig ileum for 15 minutes.

The effects of polyphenol and tannin extracts on the responses elicited by both agonists were recorded after restimulation ileum with respective agonists. The responses were recorded via a frontal writing lever on kymograph paper (Scientific and Research Instruments Ltd. England). The experiment was repeated tree times and mean percentage inhibition of both agonists contractions in the presence of test samples was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{\text{ACag} - \text{ACts}}{\text{ACag}} \times 100$$

Where ACag is the amplitude level of the contractions (cm) induced by agonists and ACts is the amplitude level of the contractions (cm) induced by tested samples. Effective doses 50 (ED<sub>50</sub>) were derived from linear curves doses-responses Atropine sulphate and papaverine hydrochloride were used as reference antispasmodic products and tested at a concentration of 40 µg/ml in organ bath (Tona *et al.*, 1999, Cimanga *et al.*, 2010).

#### 2.4. Statistical analysis

The results are reported as mean ± SD for all values. The significant differences were assessed using one-way analysis of variance (ANOVA) using SPSS software package. P values < 0.05 were considered as significant.

### 3. RESULTS AND DISCUSSION

Polyphenol and complex catechic tannin extracts were assessed for their probable capacity to inhibit contractions induced by agonists acetylcholine and depolarizing solution rich in KCL on isolated guinea-pig ileum. For good interpretation and understanding of obtained results, following criteria were adopted: 80 ≤ % Inhibition ≤ 100%: pronounced activity, 70 ≤ % inhibition < 80%: good activity, 60 ≤ % inhibition < 70%: moderate activity, 50 ≤ % inhibition < 60%: weak activity, 20 ≤ % inhibition < 50: inactive.

Against acetylcholine (ACh) effect on isolated guinea-pig ileum, results presented in Table 1 showed that polyphenol extracts from *Maprounea africana* (Ma) and *Mangifera indica* (Mi) stem bark, *Morinda morinoides* (Mm), *Psidium guajava* (Pg) and *Tithonia diversifolia* (Td) leaves produced 80% < % inhibition < 100% of contractions induced by ACh on isolated guinea-pig ileum at a tested concentration of 20 µg/ml in organ bath. For these polyphenol extracts, according to the level of their inhibition percentage produced against ACh effects, the order of activity can be established as Ma (95.40%) > Td (97.37%) > Mm (86.54%) > Mi (83.54%) > Pg (82.00%). These polyphenol extracts exerted spasmolytic activity with effective doses 50 (ED<sub>50</sub>) ranging from 11.27 to 12.85 µg/ml against the agonist ACh (Table 1) and have significant and pronounced anticholinergic effect. The effect of Ma did not shown significant difference compared to Td (p > 0.05). These samples showed pronounced antispasmodic activity. Another group of six polyphenol extracts exerted the same activity by producing a inhibition percentage between 70 and 80%. They included polyphenol extracts

from *Alchornea cordifolia* and *Cajan cajan* leaves, *Garcinia kola* stem bark, *Nauclea latifolia* and *Morinda lucida* leaves, *Bridelia ferruginea*, *Garcinia kola* stem bark, *Harugana madagascariensis* and *Nauclea latifolia* root bark exhibiting good antispasmodic activity with ED<sub>50</sub> values ranging from 12.85 to 15.25 µg/ml (Table 1).

**Table 1: Effects of polyphenol and tannin extracts on acetylcholine inducing contractions on isolated guinea-pig ileum.**

Plant names	Part used	Polyphenol extracts	ED <sub>50</sub> , µg/ml	Tannin extracts	ED <sub>50</sub> , µg/ml
<i>Alchornea cordifolia</i> (Schum et Thonn.) Müll. Arg. (Euphorbiaceae)	L	75.36±0.03	14.27±0.01	42.10±0.05	24.74±0.03
<i>Bridelia ferruginea</i> Benth. (Euphorbiaceae)	Sb	71.00±0.01	15.08±0.02	71.42±0.04	14.00±0.08
<i>Cajan cajan</i> Milsp. (Fabaceae)	L	77.90±0.03	13.84±0.05	60.20±0.06	17.61±0.03
<i>Garcinia kola</i> Heckel (Clusiaceae)	Sb	78.35±0.04	13.76±0.07	50.00±0.01	20.0±0.02
<i>Harungana madagascariensis</i> Lam. ex.Poir. (Hypericaceae)	Rb	75.00±0.05	13.34±0.01	75.51±0.02	13.24±0.04
<i>Hymenocardia acida</i> Tull. (Euphorbiaceae)	Sb	58.80±0.03	18.00±0.04	50.30±0.04	20.88±0.01
<i>Mangifera indica</i> L. (Anacardiaceae)	Sb	82.54±0.01	11.54±0.02	66.60±0.02	15.01±0.04
<i>Maprounea africana</i> Müll. Arg. (Euphorbiaceae)	Sb	97.40±0.05	11.27±0.02	73.30±0.03	13.64±0.05
<i>Morinda lucida</i> Benth. (Rubiaceae)	L	76.85±0.05	12.85±0.07	68.54±0.02	15.50±0.03
<i>Morinda morindoides</i> (Baker) Milne-Redh. (Rubiaceae)	L	86.54±0.02	11.78±0.04	76.58±0.08	12.94±0.01
<i>Nauclea latifolia</i> L. (Verbenaceae)	Rb	76.32±0.07	14.10±0.07	66.60±0.05	16.01±0.02
<i>Paropsia brazzeana</i> Baill. (Flacourtiaceae)	Rb	41.00±0.06	25.40±0.05	40.15±0.03	25.85±0.03
<i>Pentaclethra macrophylla</i> (Mimosaceae)	Sb	50.00±0.01	19.57±0.08	40.00±0.03	24.76±0.05
<i>Psidium guajava</i> L. (Myrtaceae)	L	82.00±0.03	12.35±0.05	50.00±0.05	18.57±0.04
<i>Tetracera poggei</i> Gilg. (Dilleniaceae)	L	spasmogene	ND	66.60±0.01	15.58±0.03
<i>Tithonia diversifolia</i> A. Gray (Asteraceae)	L	97.37±0.04	11.29±0.05	53.30±0.01	18.78±0.04
Atropine sulphate		100.00±0.00	4.50±0.02	-	-
Papaverine hydrochloride		100.00±0.00	3.75±0.04	-	-



The high inhibitory percentage was presented by polyphenol extracts from *G. kola* stem bark ( $78.35 \pm 0.05$ ), follow by *C. cajan* leaves ( $77.90 \pm 0.03\%$ ), *N. latifolia* and *M. lucida* leaves ( $76.32 \pm 0.07$  and  $76.85 \pm 0.05\%$ ) for which their activity did not show significant difference ( $p > 0.05$ ). These polyphenol extracts showed effective doses 50 ( $ED_{50}$ ) from 12.76 to 15.081  $\mu\text{g/ml}$ . They showed good antispasmodic activity. On the other hand, polyphenol extract from *Hymenocardia acida* and *Pentaclethra macrophylla* stem bark caused an inhibition of  $58.80 \pm 0.03$  and  $50.00 \pm 0.01\%$  of contractions induced by this agonist and exerted its activity with  $ED_{50}$  value of  $18.00 \pm 0.04$  and  $19.57 \mu\text{g/ml}$  respectively, and their effect was considered as weak. The remaining polyphenol extracts from *Paropsia brazzeana* root bark produced 41% inhibition of contractions induced by Ach and was considered as inactive, while that of *Tetracera poggei* showed spasmogenic effect i.e it increased the amplitude contractions induced by ACh on isolated organ by 115% suggesting its laxative property.

The effects of complex catechic tannin extracts (C. catechic tannins) against ACh effect on isolated organ indicated that these samples from *Bridelia ferruginea* and *M. africana* stem bark. *H. madagascariensis* root bark, *M. morindoides* leaves exerted antispasmodic activity by producing more than 75% inhibition of contractions induced by this agonist. The high percentage of activity was shown by complex catechin tannin extract from *M. morindoides* leaves ( $76.58 \pm 0.08\%$ ) followed by that of *H. madagascariensis* ( $75.51 \pm 0.02\%$ ). All complex catechic tannin exhibited antispasmodic activity with  $ED_{50}$  values ranging from 12.94 to 14.00  $\mu\text{g/ml}$  (Table 1) suggesting their good antispasmodic effect. For *M. morindoides* leaves some of its isolated antispasmodic principles are known to flavonoids rutin, quercetin and quecitrin, and irrdoids including epoxygaertneroside and gaertneroside (Cimanga et al. 2010). A moderate antispasmodic activity against this agonist was shown by complex catechic tannin from *C. cajan*, *P. guajava* and *T. poggei* leaves, *N. latifolia* root bark and *M. indica* producing  $60 < \% \text{ inhibition} < 70\%$  of contractions of isolated organe induced by this agonist with  $ED_{50}$  values ranging from 14.75 to 15.62  $\mu\text{g/ml}$  (Table 2). Those of *P. macrophylla* and *H. acida* stem bark, and *P. brazzeana* root bark exhibited weak antisapamodic activity with percentage inhibition between 42 and 56% and  $ED_{50}$  values from 17.76 to 23.75  $\mu\text{g/ml}$  (Table 1).

Moreover, against contractions induced by DRS-KCl on isolated guinea-pig ileum, results revealed that polyphenol extracts from *M. africana*, *M. morindoides*, *P. guajava* and *T. diversifolia* leaves, *M. indica* stem bark inhibited contractions induced by this agonist with a

inhibitory percentage more than 80%. The high activity was shown by polyphenol extract from *M. africana* stem bark and *T. diversifolia* (95.35±0.03% and 95.45±0.02) for which their activity did not show significant difference ( $p > 0.05$ ), followed by *M. morindoides* (84.75±0.08%). The antispasmodic activity displayed by polyphenol extract of *M. indica* stem bark compared to that of *Psidium guajava* leaves seemed to be closed (80.75±0.03 and 80.05±0.04% respectively) ( $p > 0.05$ ). They exerted pronounced antispasmodic activity with ED<sub>50</sub> values ranging from 10.55 to 12.43 µg/ml (Table 2). Polyphenol extracts from *Alchornea cordifolia*, *C. cajan* and *M. lucida* leaves, *Garcinia kola* and *Harungana madagascriensis* stem bark exhibited good antispasmodic activity by showing a inhibition percentage between 70 and 80% (Table 2). The high activity was presented by polyphenol extract of *M. lucida* (78.15±0.03%) followed by *G. kola* stem bark (76.45±0.05) and *C. cajan* (74.20±0.03%). They showed their activity with ED<sub>50</sub> values ranging from 1.85 to 15.25 µg/ml (Table 2). A moderate antispasmodic activity was displayed by polyphenol extract from *H. acida* (60.50±0.05%) while those from *P. macrophylla* stem bark, *P. brazzeana* root bark and *Tetracera poggei* leaves, caused inhibitory percentages of 52.01±0.07, 39.00±0.04, and 35.56±0.02% respectively. Polyphenol extracts from *P. brazzeana* root bark and *T. poggei* leaves produced 35 and 39% inhibition of the effect of this agonist with ED<sub>50</sub> values of 25.68 and 28.25 µg/ml, and considered as inactive.

**Table 2: Effects of polyphenol and tannins extracts on depolarizing solution rich in KCl inducing contraction of guinea-pig ileum.**

Plant names	Part used	Polyhenol extracts	ED <sub>50</sub> , µg/ml	C. catechic tannin extracts	ED <sub>50</sub> , µg/ml
<i>A. cordifolia</i>	L	70.15±0.05	15.25±0.02	40.30±0.01	23.81±0.05
<i>B. ferruginea</i>	Sb	68.20±0.04	14.79±0.02	70.65±0.02	14.35±0.05
<i>C. cajan</i>	L	74.20±0.07	13.68±0.01	65.40±0.02	15.45±0.07
<i>G. kola</i>	Sb	76.45±0.05	13.25±0.03	56.07±0.08	17.90±0.05
<i>H. madagascariensis</i>	Rb	73.30±0.06	13.75±0.04	70.20±0.04	14.36±0.08
<i>H. acida</i>	Sb	60.50±0.05	16.75±0.01	56.37±0.01	17.86±0.03
<i>M. africana</i>	Sb	95.35±0.02	10.55±0.05	70.10±0.036	14.45±0.07
<i>N. latifolia</i>	Rb	71.40±0.05	13.96±0.02	64.65±0.03	15.68±0.06
<i>M. indica</i>	Sb	80.05±0.04	12.58±0.02	68.47±0.01	14.75±0.05
<i>M. lucida</i>	L	78.15±0.07	12.85±0.03	75.05±0.03	13.45±0.07
<i>M. morindoides</i>	L	84.75±0.02	11.83±0.01	81.85±0.05	12.41±0.05
<i>P. brazzeana</i>	Rb	39.00±0.04	25.68±0.03	42.35±0.07	23.75±0.08
<i>P. macrophylla</i>	Sb	52.01±0.07	19.45±0.05	45.00±0.02	22.56±0.01
<i>P. guajava</i> L	L	80.70±0.03	12.43±0.04	65.50±0.01	15.63±0.01
<i>T. poggei</i>	L	35.56±0.02	28.25±0.07	64.20±0.06	15.62±0.04
<i>T. diversifolia</i>	L	95.45±0.02	10.55±0.02	56.43±0.04	17.95±0.08



Atropine sulphate		-	-	-	-
Papaverine hydrochloride		100.00±0.00	3.75±0.04	-	-

C. catechic: complex catechic

Regarding the effects complex catechic tannin extracts on this agonist, results indicated that, that from *M. morindoides* leaves displayed pronounced antispasmodic activity by producing 81.85±0.05 % inhibition of contractions induced by ACh with ED<sub>50</sub> value of 12.41±0.05 µg/ml. Complex catechic tannin extracts from *B. ferruginea*, *H. madagascariensis*, *M. africana* stem bark, *M. lucida* leaves exhibited good antispasmodic by causing an inhibition percentage ranging from 70 and 80% with ED<sub>50</sub> values ranging from 13.45 to 14.53 µg/ml (Table 2). The high inhibition percentage of contractions induced by this agonist was shown by complex catechic tannin extract of *M. lucida* leaves (75.05±0.03%) followed by *B. ferruginea*, *H. madagascariensis* and *M. africana* stem bark for which their activity were closed (70.65±0.05, 70.20±0.05 and 70.10±0.06% respectively). The activity of complex catechic tannin extracts from the three last medicinal did not show significant difference ( $p > 0.05$ ). These selected polyphenol and complex catechic tannins exerted a papaverine-like effect.

In a recent investigation, the antispasmodic of flavonoids extracts from these selected antidiarrhoeal medicinal plant were also previously reported (Cimanga et al., 2018b). In addition to the present results, it was clearly observed that the antidiarrhoeal properties traditionally attributed to these selected medicinal plants are partly due to the presence of polyphenolic compounds including flavonoids and tannins identified in respective plant extracts.

To evaluate whether the spasmolytic activity of these polyphenol an complex catechic tannin extracts were also mediated through Ca<sup>2+</sup> channel blockade, high concentration of K<sup>+</sup> (100 mM) was used to depolarize the preparation. Moreover, a used high K<sup>+</sup> concentration greater than 30 mM, is known to be responsible for smooth muscle contractions through opening of voltage-dependent L-type Ca<sup>2+</sup> channels, thus allowing influx of extra-cellular Ca<sup>2+</sup> causing a contractile effect (Godfraind et al., 1986) leading to considere any substance causing inhibition of the high K<sup>+</sup> induced contractions as an inhibitor of Ca<sup>2+</sup> influx (Ahmad eta l., 2012) or a Ca<sup>2+</sup> influx blocker as suggested by (Godfraind et al., 1986). Calcium antagonists constitute an important therapeutic group characterized by their dose-dependent inhibition of slow entry of this cation and their capacity for reversal of this effect by Ca<sup>2+</sup> (Fleckenstein et

al., 1977). Thus, the spasmolytic effect of these polyphenol and tannin extracts from the selected antidiarrhoeal medicinal plants in the present study, causing the relaxation of high  $K^+$ -induced concentrations, may be due to the channel blockade as also previously reported by (Ahmad *et al.*, 2012) for the fractions and extracts of *Euphorbia granualta*. Their effects may be mediated possibly through  $Ca^{2+}$  antagonist effects which can explain their therapeutic usefulness in hyperactive gut disorders, such as abdominal colic and diarrhoea as these selected medicinal plants are known to be useful in such disorders. Polyphenol and complex catechic tannin extracts used in the present study caused the relaxation of high  $K^+$  in at the tested concentration (20  $\mu$ g/ml in organ bath) and indicated the involvement of  $Ca^{2+}$  channel blocking activity in anticholinergic activity because any substance that inhibited high  $K^+$ -induced contractions is denoted CCB (calcium channel blocker) (Asifa *et al.*, 2017). The calcium channel blocking effect observed is due to the presence of tested polyphenol and complex catechic tannin extracts, as evident from phytochemical screening of these selected antidiarrhoeal medicinal species (Cimanga *et al.* 2018b), and because these constituents have been reported previously to have calcium channel blocking (Ali *et al.*, 2014).

Moreover, in KCl-induced contractions, the voltage dependent calcium channels are involved and the existence of L-type voltage dependent calcium channels in pig ileum has been reported (Naseri *et al.*, 2008). (Table 2). Periodic depolarization and repolarization of the tissues due to the influx of calcium into sarcoplasmic reticulum through voltage-dependent calcium channel are known as events responsible for spontaneous intestinal responses. According to the above observation, it has been suggested that substances that inhibit KCl-induced contractions act via blocking the channels (Vadivel *et al.*, 2017).

Based on antispasmodic activity of reference drugs, it was observed that atropine sulphate only inhibited ACh-induced contractions (100% inhibition and had no effect against DRS KCl-induced contractions of the isolated organ while papaverine hydrochloride caused 100% inhibition of contractions induced by both agonists. Thus, the tested polyphenol and complex catechic tannin extracts have a papaverine-like effect.

In addition the antispasmodic effects of these polyphenol and catechic tannin extracts was completely reversible after plentiful washing isolated guinea-pig ileum with Thyrode's solution and restimulation with both agonists suggesting that their effects were possibly not accompanied with binding to  $Ca^{2+}$  channels or/and entering to smooth muscle cells. Existence

of anticholinergic activity in these selected tested samples provided also the sound justification of their antidiarrheal properties.

Many secondary metabolites such as flavonoids (Lutterdot *et al.*, 1989, Ogongbamila *et al.*, 1990, Capasso *et al.*, 1991, Di Carlo *et al.*, 1993, de Meideiros *et al.*, 1991, Morales *et al.*, 1994), alkaloids (Calixto *et al.*, 1984, Martin *et al.*, 1993, Capasso *et al.*, 1997, el-Shafae *et al.*, 1998, de Moura *et al.*, 2002, Corea *et al.*, 2005, Zhao *et al.*, 2010, Morales *et al.*, 2013, Silva *et al.*, 2017), saponins (Corea *et al.*, 2005, Asifa *et al.*, 2017), steroids and terpenoids (Naz *et al.*, 2016, Asifa *et al.*, 2017, and tannins (Galvez *et al.*, 1993) were previously reported to exhibit antispasmodic activity in different experimental models.

Although the obtained data from this study demonstrated antispasmodic property of these selected polyphenol and complex catechic tannin extracts, their efficiencies still lower compared to atropine and papaverine used as spasmolytic reference products (Tables 1 and 2), but may be considered as new members of antispasmodic family because of their safety, tolerability and effectiveness.

#### 4. CONCLUSION

In summary, the findings from the present study suggest that polyphenol and complex catechin tannin extracts from some antidiarrhoeal medicinal plant species possess interesting antispasmodic activity. This biological activity exhibited by these chemical samples are mediated possibly through blockade of calcium channels as well as release of calcium from intracellular stores. They may be considered in part as active phytochemical for the antidiarrhoeal properties claimed by different practitioners for the different selected medicinal plants acting to treat diarrhoea by their antispasmodic activity. The found biological activity justify and support their use as raw vegetal material for the preparation of traditional remedies for the treatment of diarrhoea in children and adults in traditional medicine in Democratic Republic of Congo and other African countries.

#### REFERENCES

1. Ahmad I, Khan AU, Chaudhary BA, Janbaz KH, Uzair M, Akhtar M, Gilai AH. Antifungal and antispasmodic activities of the extracts of *Euphorbia granulate*. J Med Plants Res, 2012; 6(1): 19-23.
2. Ali N., Alam H, Khan A, Ahmed G, Shah WA, Nabi M, Junaid M. Antispasmodic and antidiarrhoeal activity of the fruit of *Rosa moschata*(J). BMC Complement Altern Med,

2014. Available from <https://bmccomplementalmed.biomedcentral.com/articles/10.1186/1472-6882-14-485>.
3. Asifa S, Mueen A, Madiha H, Alamgeer, Safur RM. Pharmacological evaluation of antispasmodic and bronchodilator effects of *Spinacia oleraceae* L. *Am J Phytothem Clin Ther*, 2017; 5(2): 1-5.
  4. Barakat BB., Muahammad I., Niaz A., Navved M., Rehmanullah. 2013. Antispasmodic potential of leaves, barks and fruits of *Zanthoxylum armatum* DC. *African Journal of Pharmacy and Phramacology*, 7(3): 685-693.
  5. Calixto JB, Yunes RA, Neto AS, Valle RM, Rae GA. Antispasmodic effects of an alkaloids extracted from *Phyllanthus sellowianus*: a comparative study with papaverine. *Braz J Med*, 1984; 17(3-4): 313-321.
  6. Capasso A, Pinto A, Mascolo N, Aurore G, Capasso F. Reduction of agonist-induced contractions of guinea-pig isolated ileum by flavonoids. *Phytother Res*, 1991; 5(1): 85-87.
  7. Capasso A, de Feo VC, Simone F, Sorrentino L. Activity-directed isolation of spasmolytic(anticholinergic) alkaloids from *Brugmania arborea*(L.) Lagerheim. *Int J Pharmacogn*. 1997, 35(1): 43-48.
  8. Chattida W., Kanokporn S., Supap S., Wararut B. 2017. In vitro analysis of antispasmodic activity of ethanolic stem bark extract of *Uvaria rufa* Blume and *Anomianthus dulcis*(Dunal J. Sinclair on excised rats' ileum. Available on doi.org:10.1007/s00580-017-2588y.
  9. Cimanga Kanyanga R., Makila Bool-Miting, F., Tona Lutete G., Kambu Kabangu, O., Vlietinck A.J., Pieters, L. Antibacterial screening of aqueous extracts of some medicinal plants and their fractions used as antidiarrheal agents in Kinshasa-Democratic Republic of Congo. *World J Pharm Pharm Sci*, 2018a; 7(5): 223-241.
  10. Cimanga KR., Makila BMF., Kambu KO., Tona LG., Vlietinck A.J., Pieters, L. *In vitro* amoebicidal activity of aqueous extracts and their fractions from some medicinal plants used in traditional medicine as antidiarrheal agents in Kinshasa-democratic-Republic of Cong0. *Eur J Biomed Pharm Sci*, 2018b; 7(5): 103-114.
  11. Cimanga K.R., Gatera G.S., Tona L.G., Kambu K.O., Vlietinck A.J., Pieters, L. 2018c. Spasmolytic activity of flavonoids extracts from some medicinal plants used as antidiarrheal agents in traditional medicine in Kinshasa-RDCongo. *World J Pharm Pharm Sci*, 2018; 7(5): 170-182.
  12. Cimanga KR., Mukenyi PNK, Kambu K.O, Tona L.G, Apers, S., Pieters L. Vlietinck A.J. 2010. The spasmolytic activity of extracts and some isolated compounds from the leaves

- of *Morinda morindoides*(Baker Milne-Redh(Rubiaceae). Journal of Ethnopharmacology, 127: 215-220.
13. Consolini AE., Berardi A., Rosella MA, Volonté M. 2011. Antispasmodic effects of *Aloysia polystachya* and *A. gratissima* tinctures and extracts are due to non-competitive inhibition of intestinal contractility induced by acetylcholine and calcium. Revista Brasileira de Farmacognosia, 21(5): 1-16.
  14. Corea G, Fattorusso E, Lanzotti V, Capasso R, Izzo AA. Antispasmodic saponins from bulbs of red onion, *Allium cepa* L. var *Tropea*. J Agr Food Chem, 2005; 53(4): 935-940.
  15. de Medeiros CLC, Thoma G, Mukherje R. The source of Ca<sup>2+</sup> for the spasmolytic actions of longicaudine, a bisindole alkaloid isolated from *Strychnos trinevis*(Vell.) Mart.(Loganaceae). Phytother Res, 1991; 5(1): 24-28.
  16. De Moura NF, Morel AF, Dessoy EC, Zantta N, Burger MM, Ahlert N, Porto GP, Baldisserotto B. Alkaloids, amides and antispasmodic activity of *Zanthoxylum hyemale*. Planta Med, 2002; 68(6): 534-538.
  17. Di Carlo G, Autore G, Izzo AA, Maiolino P, Mascolo N, Viola P, Diurno MV, Capasso F. Inhibition of intestinal motility and secretion by flavonoids in mice and rats: structure-activity relationship. J Pharm Pharmacol, 1993; 42(12): 1054-1059.
  18. el-Shafae AM. A pyranocoumarin and two alkaloids(one with antispasmodic effect) from *Citrus delicosa*. Die Pharmazie, 1998; 53(9): 640-643.
  19. Fleckenstein A. Specific pharmacology of Ca<sup>++</sup> in myocardium cardiac pacemakers and vascular smooth muscle. Rev Pharmacol Toxicol, 1977; 17(1): 149-166.
  20. Galvez J, Cresspo M, Zarzuelo EE, de Witte P, Spenssens C. Pharmacological activity of procyanidin activity and effect on isolated guinea-pig ileum. Phytother Res, 1993; 7(1): 25-27.
  21. Gilani AH, Mandukhail SR, Iqbal J, Yasinzi M, Aziz N, Khan A, Najeeb-er-Rehman. Antispasmodic and vasodilator activities of *Morinda citrifolia* root extract are mediated through blockade of voltage dependent calcium channels BMC Complement Altern Med, 2010; 10(2): 1-9.
  22. Godfraind T, Miller R, Wibo M. Calcium antagonism and calcium entry blockade. Pharm Rev, 1986; 38(3): 321-416.
  23. Irshad A., Arif-Ullah K., Bashir AC, Khalid H.J., Muhammad U., Muhammad A., Anwarul-Hassan G. Antifungal and antispasmodic activities of the extracts of *Euphorbia granulata*. Journal of Medicinal Plants Research, 2012; 6(1): 19-23.

24. Harborne JB. Phytochemical Methods. A Guide to Modern Techniques of Plant analysis. Chapman and Hall, London, 1998.
25. Lutterdot GD, Inhibition of gastrointestinal release of acetylcholine by quercetin as a possible mode of action of *Psidium guajava* leaf extracts in the treatment of acute diarrhoeal disease. J Ethnopharmacol, 1989; 25(2): 239-247.
26. Martin ML, Diaz MT, Montero MJ, Prieto P, San Roman L, Cortes D. Antispasmodic activity of benzyloquinoline alkaloids analogous to papaverine. Planta Med, 1993; 59(1): 63-67.
27. Mendel M, Chlopecka M, Dziekan N, Karlik W. Antispasmodic effect of selected *Citrus* flavonoids on rat isolated jejunum specimens. Eur J Pharmacol, 2016; 791(4): 640-646.
28. Morales MA, Ahumada F, Castillo E, Burgos R, Christen P, Bustos V, Muñiz O. Inhibition of cholinergic contractions of rat ileum by tropane-type alkaloids present in *Schizanthus hookeri*. Naturfosh, 2013; 68(2): 203-209.
29. Morales MA, Tortoriello J, Meckes M, Paz D, Luzoya X. Calcium antagonist effect of quercetin and its relation with spasmolytic properties of *Psidium guajava* L. Arch Med Res, 1994; 25(1): 17-21.
30. Naseri MKG, Yahavi H, Arabian M. Antispasmodic of Onion(*Allium cepa* L.) pelle extract on rat ileum. Iranian J Pharm Res, 2008; 7(2): 155-159.
31. Naz SB, Chaudhary M, Reman MS. Dual receptor blocker mechanism attributes smooth muscle relaxant of *Polypodium vulgare* Linn. Bangladesh J Pharm, 2016; 11(4): 414-420.
32. Ogongbamila FO, Sameulson G. Smooth muscle relaxation flavonoids from *Alcornea cordifolia*. Acta Pharm Nordica, 1990; 2(4): 421-422.
33. Prasanna PG., Ajit SK., Nagesh HA., Riyaz AO., Rohit RB., Bhargav RH., Birudev BK. 2013. *In-vitro* antispasmodic activity analysis of methanolic leaves extract of *Lantana camara* Linn. on exised rat ileum. J Pharmacogn Phytochemistry, 2(3): 66-71.
34. Silva FL, da Silva LV, Silva JM, Marcoli LSA, Nouailhetas VLA, Yoshida M, Vendramini PH, Eberlin MN, Barobosa-Filho JM, Moreno PRH. Antispasmodic activity from *Serjania carasana* fractions and their safety. Rev Bras Farmacogn, 2017; 27(3): 1-15.
35. Tona L., Kambu K., Mesia K., Cimanga K., Apers, S., De Bruyne T., Pieters L., Totté J., Vlientick, AJ. 1999. Biological screening of traditional preparations from some medicinal plants used as antidiarrhoeal in Kinshasa. Congo. Phytomedicine, 6(1): 59-66.
36. Trease GE, Evans WC. Pharmacognosy, WB Saunders, Philidelphia, USA, 1996.



37. Vadivel K, Kumar GS, Babu SM. *Ex vivo* antispasmodic activity of aqueous extract of flowers of *Muntingia calabura* Linn. On excised rabbit's jejunum. *Pharmacognosy Res*, 2017; 9(3): 301-303.
38. Zhao M, Xian YF, Ip SP, Fong HHS, Che CT. A new weakly antispasmodic protoberberine alkaloid from rhizome *Coptidis*. *Phytother Res*, 2010; 24(9): 1414-1416.