Scholars Academic Journal of Pharmacy (SAJP) **ISSN 2320-4206 (Online)**
Sch. Acad. J. Pharm., 2014; 3(1): 66-72 **ISSN 2347-9531 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Research Article

Calcium antagonist of n-butanol fraction (BuF) from the stem bark of *Terminalia superba* Engl. et Diels (Combretaceae) on rabbit duodenum

Nomane Bernard Goze[1], Kouakou Léandre Kouakou[1], Nahounou Mathieu Bléyéré[1*], Brou AndréKonan[2], Kouao Augustin Amonkan[2], Kouakou Jean Claude Abo [3], Ehouan Etienne Ehilé [1]

[1]Laboratory of Physiology, Pharmacology and African Pharmacopoeia of UFR-SN, University of Nangui Abrogoua, Côte d'Ivoire.

[2]Laboratory of Pharmacology and Nutrition of UFR-Biosciences, University of Felix Houphouet Boigny, Cocody, Côte d'Ivoire.

[3]Laboratory of Animal Physiology of UFR-Biosciences, University of Felix Houphouet Boigny, Cocody, Côte d'Ivoire.

***Corresponding author**
Dr Mathieu Nahounou BLEYERE, PhD,
Email: bleyere@yahoo.fr, chridandre@gmail.com

Abstract: The Objective of the present study was to investigate the pharmacological effects and the possible mechanism (s) that mediated the effects of the organic fraction from n-butanol-water partiton (50:50 v/v) of the stem bark of *Terminalia superba* and to screen for the presence of phytochemical constituents which can be responsible for these observations. Organic n-butanol fraction (BuF) from the stem bark of *Terminalia superba* was evaluated for its capacity to induce spasmolytic effect on spontaneous contraction of isolated rabbit duodenum as well as for its possible calcium blocking actions. Pieces of rabbit duodenum (3cm) were mounted in an organ bath containing air bubbled Mac Ewen solution with 1g initial tension. The non cumulative concentrations of BuF exhibited a significant ($p<0.05$) relaxation on the spontaneous contraction of isolated rabbit duodenum for concentrations ranging from 0.02 to 0.08 mg/ml. The pre-contracted duodenum by ACh ($2.20x10^{-6}$mg/ml), KCl ($2.43 x10^{-6}$mg/ml) and $BaCl_2$ ($2.30 x10^{-6}$mg/ml) were relaxed by the non cumulative concentrations (0.02, 0.04 and 0.08 mg/ml) of BuF ($p<0.05$). In high potassium ($2.43 x10^{-6}$mg/ml) Ca^{2+}-free Mac Ewen solution, non cumulative concentration of $CaCl_2$ ($2.02 x10^{-3}$µg/ml) induced duodenum contractions. However, the non cumulative concentrations of BuF (0.02, 0.04 and 0.08 mg/ml) reduced these $CaCl_2$-induced contractions concentration-dependently ($p<0.05$). These results suggest that BuF antispasmodic effect on rabbit duodenum may be due to blockade of voltage dependent calcium channels. Tannins, flavonoids, quinones, saponins, reduced sugar, sterols and polyterpenes were found out as major active constituents of the tested extract. Some of these phytochemical constituents such as tannins, flavonoids, saponins, sterols and polyterpenes which are known for their antispasmodic effect may explain the relaxant actions of BuF.
Keywords: Antispasmodic, *Terminalia superba*, rabbit, duodenum.

INTRODUCTION

Terminalia superba Engl. et Diels (Combretaceae) commonly called «fraké» or «limbo» grows from France-Guinea, Congo, Cameroon, Angola to Democratic Republic of Congo [1]. In Côte d'Ivoire, this plant grows in the same place with *Triplochiton scleroxylon* (Sterculiceae) generally called «samba ». *Terminalia superba* is widely used for the treatment of various illnesses in folk medicine. The stem barks of the plant are used to heal diseases including abdominal pains, diabetes, diarrhoea, peptic ulcers, female infertility, headache, dysentery, bacterial infections and general fatigue [2-3-4-5-6-7]. Analgesic, antibacterial and endothelium independent vasorelaxant properties were found out [8-9-10]. Although *Terminalia superba* is used as antigastrointestinal disorders agent in folk medicine, no or few scientific studies were undertaken to explain its actions and usefulness as antispasmodic. The aim of the present study was to investigate the pharmacological effects and the possible calcium antagonist role of the n-butanol organic fraction from the stem bark of *Terminalia superba* and to screen for the presence of phytochemical constituents which can be responsible for these observations.

MATERIALS AND METHODS

Plant material

The stem barks of *Terminalia superba* were collected locally from the forest of Ebillassokro village in the East of Côte d'Ivoire in December 2009. Taxonomical identification of the stem barks was established by Professor Aké-Assi Laurent from the National floristic Centre of University of Felix Houphouet Boigny, Cocody- Abidjan, Côte d'Ivoire, voucher n° 2456, *Terminalia superba* Engl. et Diels in June 4, 1954 ; n° 4207 in March 26, 1957 ; n° 10477, February 26, 1969 and n° 416 in April 03, 1974 of Côte d'Ivoire national herbarium.

Preparation of the extracts

The stem barks were dried under shade and powdered with a machine (mark RETSCH, type SM 100, Germany). The extraction process was implemented according to Ivorian researchers' method [11]. One hundred grams (100g) of the stem barks powder were macerated during 24 hours in 1l ethanol-water (70:30 v/v) for 3 times until complete exhaustion. The mixtures were filtered (Whatman n°1) and concentrated under reduce pressure using a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 45°C. The concentrated extracts were stored in dessicators at 45°C. Ten grams (10g) of the dried hydroethanol extracts 70% were then partitioned separately in *n*-butanol (Bu), chloroform (Ch), ethylacetate (Ea) and hexane (Hex)-water (50:50 v/v). Two fractions were obtained from each partition, the organic and the aqueous fractions after decantation, solvent evaporation and storage in dessicators at 45°C respectively. Organic fractions obtained from these partitions were BuF (3.218g), FCh (3.218g), EaF (3.218g), HexF (3.218g) and the aqueous fractions were $Bu_{aq}F$ (6.782g), $Ch_{aq}F$ (6.782g), $Ea_{aq}F$ (6.782g), $Hex_{aq}F$ (6.782g). The concentrations to be tested were prepared extemporaneously by dilution in the Mac Ewen physiological solution (mM): NaCl, 130; KCl, 2.5; $CaCl_2$, 2.4; NaH_2PO_4, 1.18; $NaHCO_3$, 11.9; $MgCl_2$, 0.24; glucose, 2.2 pH=7.4. The *n*-butanol organic fraction (BuF) was found to be the most interesting fraction because it produced more than 80% ($p<0.05$) inhibition of contractions induced by rabbit duodenum during the preliminary tests and was retained for pharmacological and phytochemical tests.

Animals

Ten (10) rabbits (*Oryctologus cuniculus*) of either sex weigthing 2 ±0.2 kg were selected for the experiments. They were bred in the Animal House of the Laboratory of Physiology, Pharmacology and Pharmacopoeia of UFR Sciences of Nature at the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University of Nangui Abrogoua (Abidjan, Côte d'Ivoire). The animals were deprived of food for 24 hours prior to the experiments.

Drugs

Acetylcholine (ACh) was purchased from Prolabo (France). Barium chloride ($BaCl_2$), Calcium chloride ($CaCl_2$), Potassium chloride (KCl) and solutes of Mac Ewen solution were purchased from Sigma (USA).

Duodenum preparations and experimental protocol

All animals were sacrificed by stunning [12-13]. After laparatomy incision, a portion of duodenum (taken within a distance of 5 cm from pylorus) was removed and washed with Mac Ewen solution. Lengths of approximately 3 cm were put in a dish containing the oxygenated physiological solution of Mac Ewen. A selected duodenum strip was placed between two stainless steel hooks vertically in an isolated organ bath thermostated at a temperature of 37°C. The lower hook was fixed to the bottom of the organ bath (150 ml) and the upper one was connected to a C.F. PALMER kymograph transducer [12-13] which transmitted the movements on paper. The initial tension was 1g throughout the experiments. After 60 min, the time necessary for stabilization of the contractile movements, the concentrations to be tested were injected directly into the isolated organ bath containing the oxygenated physiological solution of Mac Ewen. Then the following experiments were performed.

BuF was prepared as 5 mg/ml. A single volume in geometric progression (0.5, 1.0, 1.5, 2.0 and 2.5 ml) of BuF was added in non cumulative manner to the organ bath (150 ml) to obtain concentration-dependent inhibitory effect. The final concentrations of the extract were determined as function of the final volume in the organ bath. The duodenum contractions were induced by final bath concentrations of ACh (2.204×10^{-6} mg/ml), $BaCl_2$ (2.303×10^{-6} mg/ml) or KCl (2.435×10^{-6} mg/ml) and when the plateau was reached, the extract was added non cumulatively to the organ bath, giving final concentrations ranging from 0.02 to 0.08 mg/ml. To show whether the spasmolytic activity of the active fraction was done via a calcium channel blockade, the duodenum was allowed to stabilize in normal Mac Ewen solution, and then was replaced by a Ca^{2+}-free Mac Ewen solution for 30 min. This solution was replaced with final bath concentration of K^+ rich (2.435×10^{-6} mg/ml) and Ca^{2+}-free Mac Ewen solution. Following an incubation period of 30 min and after confirmation of no spontaneous contractions of duodenum, Ca^{2+} was added non cumulatively to the organ bath giving final bath concentrations varying from 7.9×10^{-9} to 7.9×10^{-5} mg/ml to obtain control concentration-response curves of Ca^{2+}. Then, the same procedure was repeated with a single final bath concentration of $CaCl_2$ (2.02×10^{-3} µg/ml) and when the plateau of contractions was reached, final concentrations of BuF (0.02, 0.04, 0.08 mg/ml) were administered. All the concentrations in the results are final bath concentrations.

Phytochemical screening

The organic fraction of *n*-Butanol extract from the stem barks of *Terminalia superba* was screened for the presence of polyphenols, tannins, flavonoids, polyphenols, saponins, alkaloids, sterols and ployterpenes, reduced sugar, proteins, coumarines and quinones. Detection of these constituents was carried out as described by some authors [14]. This experiment was repeated 3 times (n=3).

Data analysis

All values were expressed as mean ± standard error on the mean (m±sem). Statistical analysis and

graphics were carried out using the software GraphPad Prism 5.01 (San Diego California, USA). The significance of the differences observed between the concentrations was achieved by analysis of variances (ANOVA) of the multiple test of comparaison of Tukey-Kramer. The differences between the concentrations were considered statistically significant when $p<0.05$. The plateau of the contraction caused by each spasmogen (ACh, KCl, $BaCl_2$ and $CaCl_2$) in the absence of the extract was considered as 100 % contraction.

RESULTATS

Spasmolytic effects of BuF on isolated rabbit duodenum spontaneous contractions

BuF, at non cumulative concentrations ranging from 0.02 to 0.08 mg/ml, caused a concentration-dependent and significant ($p<0.05$) decrease of the amplitude and the contractile force of rabbit duodenum contractions. The effects of BuF appeared about 3 min after administration of the substance. The amplitude diminished from 300 ± 40 to 5 ± 0.1mg. The contractile force decreased from 37 ± 1 to 820 ±30 mg under baseline. These decreases corresponded to variations from 17.95± to 100 % for the amplitude and from 4.50 ±3.1 to 100 % for the contractile force. The EC_{50} values determined graphically (Fig.1) were 0.043 mg/ml (BuF) for the amplitude and 0.055 mg/ml for the contractile force. The spasmolytic effects of BuF were reversible and the spontaneous activity returned to normal after washing the preparations.

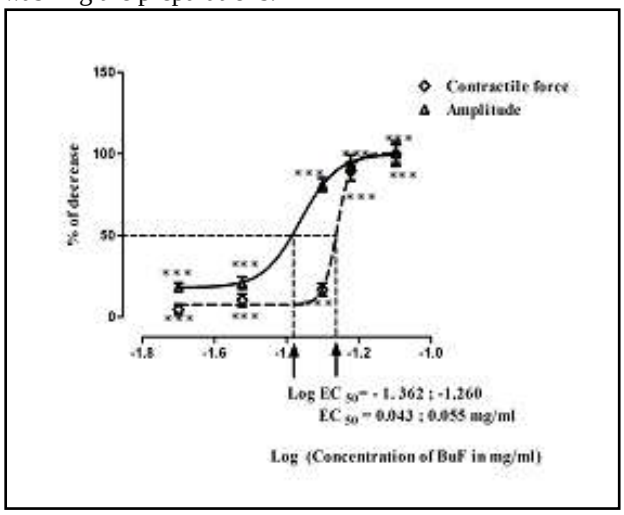

Fig. 1. Concentration-response curve for BuF on rabbit duodenum spontaneous contractions

BuF elicited significant decrease of spontaneous contractions of isolated rabbit duodenum.

Significant differences: ***$p<0.0001$, n=5 (one way ANOVA)

Effect of BuF on duodenum contractions induced by ACh, KCl and $BaCl_2$

Non cumulative concentrations of BuF (0.02, 0.04, 0.08 mg/ml) reduced significantly ($p<0.05$) and concentration-dependently after 2.5 min the increase of the amplitude and the force of muscle contraction of rabbit duodenum induced by ACh (2.204 $x10^{-6}$ mg/ml), KCl (2.435 $x10^{-6}$ mg/ml) and $BaCl_2$ (2.303 $x10^{-6}$ mg/ml). The decrease of the amplitude varied from 400 ± 30 mg to 50 ± 1mg (ACh-induced contractions), 550 ± 40 mg to 110 ± 10 mg (KCl-induced contractions), and 500 ± 20 mg to 100 ± 70 mg ($BaCl_2$-induced contractions). These decreases corresponded to variations of 93.12 ± 2.88% to 12.18 ± 4.11 % (ACh-induced contractions), 91.40 ± 3.21% to 19.18 ± 3.75 % (KCl-induced contractions), and 83.25 ± 3.49% to 16.16 ± 3.13 % ($BaCl_2$-induced contractions). The contractile force diminished from 1390 ± 30 to -170 ± 50 mg (ACh-induced contractions), 1620 ± 80 to 180 ± 10 mg (KCl-induced contractions), 2120 ± 300 to 320 ± 50 mg ($BaCl_2$-induced contractions) corresponding to variations from 85.22 ± 2.41 to -10.76 ± 2.18 % (ACh-induced contractions), 95.50 ± 2.89 to 10.43 ± 1.26 % (KCl-induced contractions), 75.65 ± 2.53 to 11.58 ± 4.27 % ($BaCl_2$-induced contractions) for concentrations ranging from 0.02 to 0.08 mg/ml respectively (Fig.2-A, B and C).

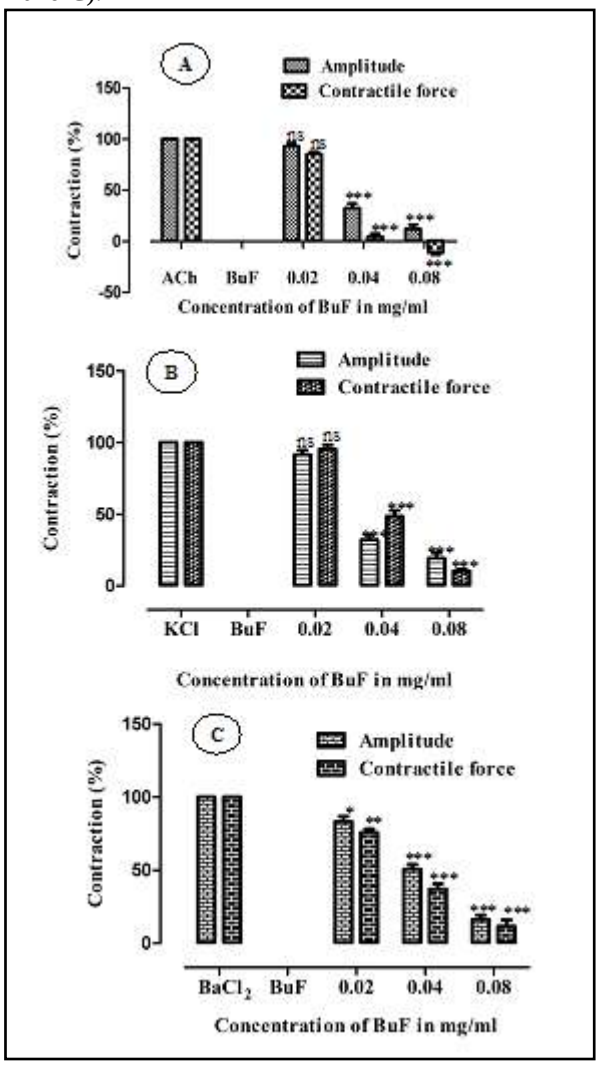

Fig. 2. Effects of BuF on contractions induced by different spasmogenic substances

Non cumulative concentrations of BuF reduced the contractions induced by ACh at 2.204 $x10^{-6}$ mg/ml

(**Fig2-A**), KCl at 2.435 x10^{-6} mg/ml /ml (Fig. 2-B**)** and $BaCl_2$ at 2.303 x10^{-6} mg/ml /ml (Fig. 2-C) on the rabbit duodenum. The differences between spasmogenic effect of each spasmogen in the absence and in the presence of the extract are shown (*p<0.05, **p<0.001, ***p<0.0001, n=5 (one way ANOVA))

Effect of BuF on $CaCl_2$-induced duodenum contractions

The contractile response of the duodenum to $CaCl_2$ solution is shown in Fig. 3-A. In the calcium free high potassium medium, rabbit duodenum contractions were reduced and vanished. The addition of $CaCl_2$ in the range of concentrations from 7.9x10^{-9} to 7.9x10^{-5} elicited an increase of the amplitude (500 ± 10 mg to 1100 ± 500 mg) and the basal tone (10 ± 4 to 2510 ± 120 mg). The EC_{50} values determined were 1.628 x10^{-3}µg/ml and 2.406x10^{-3}µg/ml respectively (Fig. 3-B).

A single concentration of $CaCl_2$ (2.02 x10^{-3} µg/ml) was used in the calcium free high potassium medium to achieve the dose response effects of BuF. After the stabilization of the effects of $CaCl_2$ (2.02 x10^{-3} µg/ml), BuF was added in the same medium. BuF reduced the $CaCl_2$-induced contractions. Indeed, after 3.5 min, a significant (p<0.05) concentration-dependent decrease of the amplitude and the force of muscle contractions was observed (Fig.3-C) with maximum inhibition at 0.08 mg/ml of BuF. The values varied from 400 ± 50 mg to 80 ± 2 mg (for amplitude), 930 ± 80 to -300 ± 10 mg (for muscle basal tone) corresponding to 92.87 ± 3.96 to 0.2 ± 0.01 % (for amplitude) and 85.03 ± 3.78 to -27.38 ± 3.55 % (for contractile force) for extract concentrations ranging from 0.02 to 0.08 mg/ml (Fig.3-D).

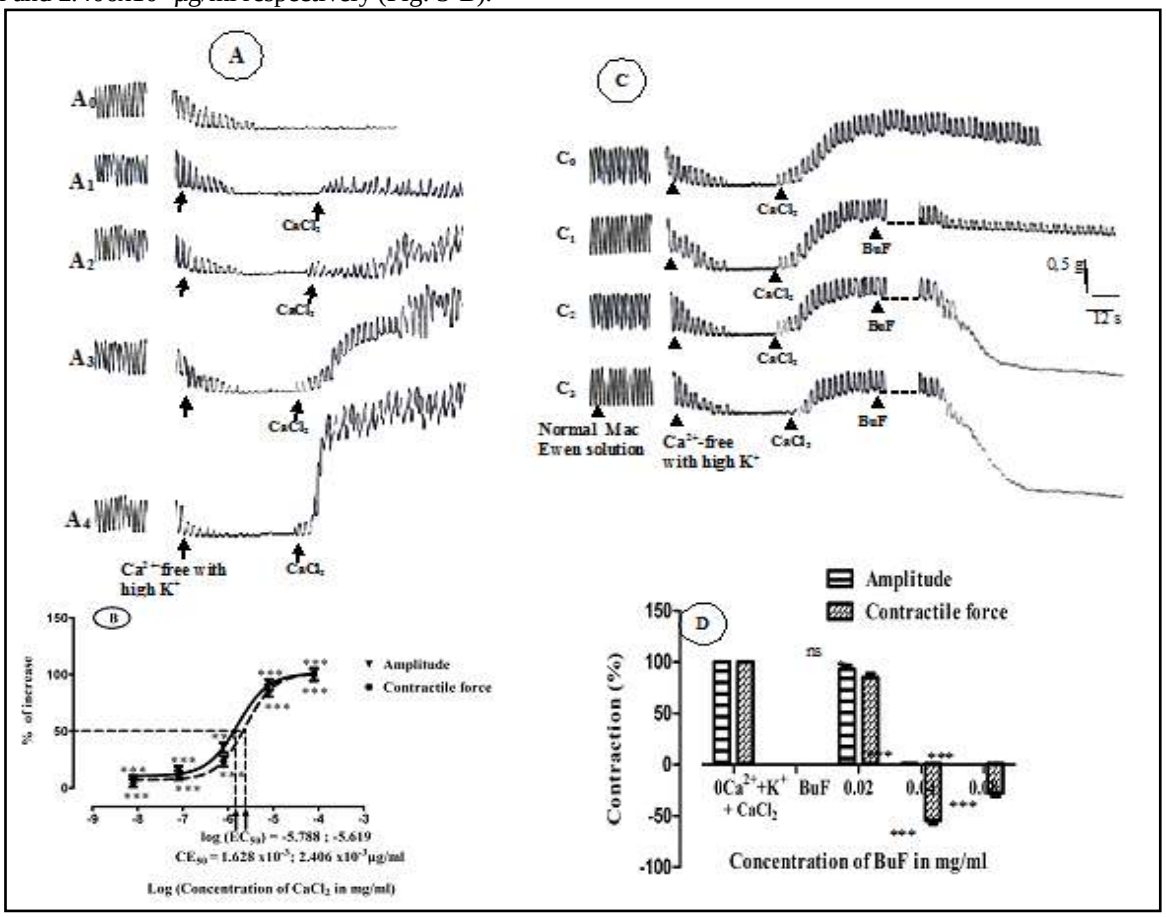

Fig. 3. Effects of BuF on $CaCl_2$-induced contractions on rabbit duodenum

Fig. 3-A: Spasmogenic effect of $CaCl_2$ on the rabbit duodenum spontaneous contractions in Ca^{2+}-free high K^+ Mac Ewen solution-depolarized solution

Control (A_0) ; effects of $CaCl_2$ at 7.9x10^{-8} (A_1); 7.9x10^{-7} (A_2); 7.9x10^{-6} (A_3) and 7.9x10^{-5} (A_4) mg/ml in Ca^{2+}-free with high K^+ Mac Ewen solution-depolarized rabbit duodenum are indicated by the second arrow.

Fig. 3-B: Concentration-response curve for $CaCl_2$ on the rabbit duodenum spontaneous contractions.

Fig. 3-C: Spasmogenic effect of non cumulative concentration of $CaCl_2$ (2.02 x10^{-3} µg/ml) on Ca^{2+}-free with high K^+ Mac Ewen solution-depolarized rabbit duodenum in the absence (C_0) and in the presence of different concentrations of BuF. (C_1: 0.02; C_2: 0.04 and C_3: 0.08 mg/ml)

Fig. 3-D: Effect of non cumulative concentrations of BuF(0.02; 0.04 and 0.08 mg/ml) on the rabbit duodenum contractions induced by $CaCl_2$ (2.02 x10^{-3} µg/ml) in Ca^{2+}-free with high K^+ Mac Ewen solution. The differences between spasmogenic effect of $CaCl_2$ in the absence and in the presence of the extract are shown (*p<0.05, ***p<0.0001, n=5 (one way ANOVA))

Phytochemical studies

Phytochemical screening of the tested extract showed positive results for Polyphenols**,** Tannins, Flavonoids, Quinones, Reduced sugar, Sterols and Polyterpenes and negative results for alkaloids, saponins, proteins and coumarines (Table 1).

Table 1. Phytochemical screening of BuF

Constituents	Reagents	BuF
Polyphenols	$FeCl_3$ test	+
Tannins	Stiasny test	+
	$FeCl_3$ test	+
Flavonoids	Cyanidine test	+
Quinones	Borntraëger test	+
Alkaloids	Bouchardat test	-
	Dragendorff test	-
	picric Acid test	-
Saponins	Frothing test	-
Sterols polyterpenes	Liebermann test	+
Reduced sugar	Tollens test	+
Proteins	Biuret test	-
Coumarines	reaction on the lact cycle	-

-: Negative reaction; +: Positive reaction

Phytochemical tests of BuF revealed the presence of Polyphenols, Tannins, Flavonoids, Quinones, Reduced sugar, Sterols and Polyterpenes. n=3.

DISCUSSION

BuF induced a concentration-dependent decrease of amplitude and force of muscle contractions of isolated rabbit duodenum when it was applied in a range of concentrations from 0.02 to 0.08 mg/ml. Furthermore, after washing, this inhibitory activity was totally reversible suggesting that these effects are membrane mediated. This was demonstrated with fruit hydroalcoholic extract of *Anethum graveolens* (Umbelliferae) on the rat ileum contractions [15]. Moreover, our results are similar to what was obtained with extracts of *Piper nigrum* (Labiatae) leaf, *Buddleja scrodiodes* (Scophulariaceae) and *Buddleja perfoliata* (Scophulariaceae) aerial parts and *Morinda morindoides* (Rubiaceae) which were also found to induce spasmolyitc effects on the rat ileum, rabbit and guinea pig intestine and on isolated guinea pig ileum respectively [16-17-12].

In order to elucidate the mechanism underlying the inhibitory effects of the plant extract, BuF as calcium antagonist was hypothesized and investigated. It had been reported that the disturbance of L-type Ca^{2+} channel activity was one of the causes of intestinal motility decrease [18].

This study revealed that BuF can elicit spasmolytic effects on ACh, KCl and $BaCl_2$-induced contractions on isolated rabbit duodenum. The ability of the extract to inhibit ACh, $BaCl_2$ and KCl-induced contractions may indicate that the spasmolytic compounds present in BuF extract are not specific receptor antagonists. It is known that these chemicals have the capacity to mobilize either intracellular or extracellular calcium or both of them and show that BuF could act on those calcium components. KCl is often used as a tool to bypass G-Protein Coupled Receptor (GPCR) stimulation and activates smooth muscle by changing the K^+ equilibrium potential and clamping membrane potential at some value above the resting level [19]. It is well known that depolarization induced by high potassium concentration activates L-type voltage dependent calcium channels ($VDCC_S$) [20]. ACh, a neurotransmitter of the parasympathetic nervous system induces contractions by activation of muscarinic receptors which in turn, increase the intracellular calcium through Inositol Triphosphate (IP_3) and also by facilitating the inflow of extracellular calcium through the receptor operated calcium channel [21-22-23-24]. In addition, $BaCl_2$ as non selective potassium channel blocker induces depolarization and contraction in smooth muscles possibly by promoting calcium release from intracellular pools [18-25].

BuF induced its spasmolytic effect probably by blocking L-type voltage dependent calcium channels. This suggestion is supported by the existence of L-type $VDCC_S$ in the intestinal smooth muscle [26]. The contractions induced by ACh, $BaCl_2$ and KCl are dependent on the entry of Ca^{2+} into the cells through $VDCC_S$, therefore a substance which can inhibit ACh, $BaCl_2$ and KCl-induced contractions is considered to be a calcium channel blocker [27]. However, it is also possible that BuF could relax the intestinal smooth muscle by interfering with calcium transport across membrane or blocking Inositol Triphosphate mediated Ca^{2+} from internal stores.

In order to confirm the role of $VDCC_S$ blockade as a possible mechanism that mediated the relaxant effect of BuF, depolarization of the duodenum preparation in Ca^{2+}-free high K^+ Mac Ewen solution was carried out. The results indicated that BuF significantly inhibited in a concentration-dependent manner the $CaCl_2$-induced contractions of isolated rabbit duodenum. These results corroborated that relaxation observed was primarily mediated by the impediment of extracellular calcium influx via $VDCC_S$ as it is done by organic calcium channel antagonists such as verapamil, which inhibits markedly the entry of calcium through $VDCC_S$ [16]. However, the inhibitory effect clearly indicates the involvement of $VDCC_S$ blockade in the spasmolytic effects of BuF. The results revealed that BuF extract contained tannins, flavonoids, quinones, saponins, reduced sugar, sterols and polyterpenes. Some of these phytochemical constituents such as tannins, flavonoids, saponins, sterols and polyterpenes which are known for their antispasmodic

effect may explain the relaxant actions of BuF [28-29-30].

CONCLUSION

The organic n-butanolic fraction from the stem bark of *Terminalia superba* (BuF) contains spasmolytic constituents mediating their effect probably through blockade of Ca^{2+} influx, which may justify the traditional use of the plant. Therefore, further experiments are required to isolate, purify and characterize the active constituent and to elucidate the exact mechanism of action.

ACKNOWLEDGEMENTS

The authors are thankful to all other members of Laboratory of Physiology, Pharmacology and Phytotherapy for their encouragement during these investigations.

REFERENCES

1. Aubréville A; La flore forestière de la Côte d'Ivoire. Deuxième édition révisée, Tome 3ème, Publication n° 15 du centre forestier tropical, 1959.
2. Adjanohoun ES, Aké Assi L; Contribution au recensement des plantes médicinales de Côte d'Ivoire. CRES, Univ., Centre National de Floristique, Côte d'Ivoire, 1979.
3. Aké Assi L; Flore de la Côte d'Ivoire: Etude descriptive et biogéographique avec quelques notes ethnobotaniques. Thèse Fac. Sc. Univ. Abidjan, 3 tomes, 6 volumes, Côte d'Ivoire, 1984.
4. Adjanohoun E, Ahyi M, Ake-Assi L, Elewude JA, Dramane K, Fadoju SO, Gbile ZO, Goudole E, Johnson C, Keita A, Morakinyo O, Ojewole J, Olatunji AO, Sofowora EA; Traditional medicine and Pharmacopoeia: Contribution to ethnobotanical floristic studies in Western Nigeria, Pub. Organization of African Unity, Scientific Technical and Research Commission Lagos, Nigeria, 1991.
5. Zirihi GN ; Contribution au recensement, à l'identification et à la connaissance de quelques espèces végétales utilisées en médecine traditionnelle chez les Bété du Département d'Issia, Côte-d'Ivoire. Thèse de Doctorat 3e Cycle Fac. Sc. Abidjan, Côte d'Ivoire, 1991
6. Hutchings A, Scott AH, Lewis G, Cunninghan A; Zulu Medicinal Plants, An Inventory. University of Natal Press, Pietermarizburg, South Africa, Jaypee Brothers: New Delhi, India, 1996.
7. Van Wyk BE, Van Oudtshoorn B, Gericke N; Medicinal plants of South Africa. Briza Publications, Pretoria, South Africa, 1997.
8. Dongmo AB, Galba JB, Tsabang N, Kamanyi A; Analgesic activities of the stem bark extract of *terminalia superba* engl. et diels **(**Combretaceae). Pharmacologyonline. 2006; 2: 171-177.
9. Bouboutou OHR, Loukou GY, Tako-Louisor D; Evaluation de l'activité antidiarrhéique de quelques plantes utilisées en Afrique et en Côte d'Ivoire. Revue méd Phar. Afric, 1995; 9 (1): 31-42.
10. Dimo T, Laurent F, Rakotonirina SV, Tan PV, Kamtchouing P, Dongo E, Cros G; Methanol extract of *Terminalia superba* induces endothelium independent relaxation of rat thoracic aorta. Pharmazie, 2006; 61(5): 470-473.
11. Zirihi GN, Kra AM, Bahi C, Guédé Guinan F ; Plantes médicinales immunostimulantes: critères de sélection, techniques rapides d'extraction des principes actifs et méthodes d'évaluation de l'activité immunogène. Pharm. Afrique, 2003; 17: 131-138.
12. Cimanga RK, Mukenyi PNK, Kambu OK, Tona GL, Apers S, Totté J, Pieters L, Vlietinck AJ;The spasmolytic activity of extracts and some isolated compounds from leaves of *Morinda morindoides* (Baker) Milne-Redh. (Rubiaceae). J Ethno., 2010; 127: 215-220.
13. Udia PM, Braide VB and Owu DU; Antispasmodic and spasmolytic effects of methanolic extract from seeds of garcinia kola on isolated rat small intestine. *Nigerian Journal of Physiological Sciences*, 2009; 24 (2): 111 -116.
14. Bekro Y, Bekro J, Boua BB, Tra Bi FH, Ehilé EE; Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend et Zarrucchi (Caesalpiniaceae). Sci Nat (Côte d'Ivoire), 2007; 2 (4): 217-225.
15. Gharib-Naseri MK, Heidari A; Antispasmodic effect of *Anethum graveolens* fruit extract on rat ileum. Int J Pharmacol., 2007; 3: 260-264.
16. Cortés AK, Delgadillo AJ, Hurtado M, Dominguez-Ramirez, Medina JR, Aoki K; The antispasmodic activity of *Buddleja scrodiodes* and *Buddleja perfoliata* on isolated intestinal preparations. Biol Pharm Bull., 2006; 29: 1186-1190.
17. Gharib-Naseri MK, Yahyavi H; Antispasmodic effect of *Piper nigrum* Fruit Hot Water Extract on rat ileum. Pak. J Biol Sci., 2008; 11: 1492-1496.
18. Liu S, Hu HZ, Ren J, Gao C, Gao N, LIN Z, Xia Y, Wood JP; Pre- and postsynaptic inhibition by nocioceptin in guinea pig small intestinal myenteric plexus *in vitro*. Am J Physiol Gastrointest Liver Physio., 2001; 281: G237-G246.
19. Ratz PH, Berg KM, Urban NH, Miner AS; Regulatin of smooth muscle calcium sensitivity: KCl as a calcium sensitizing stimulus. Am J Physiol Cell Physiol., 2005; 288: 769-783.

20. Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K, Harada K, Miyamoto S, Nakazawa H, Won KJ, Sato K; Calcium movements, distribution and function in smooth muscle. Pharmacol Rev., 1997; 49: 157-230.
21. Eglen RM, Hedge SS, Waston N; Muscarinic receptor subtypes and smooth muscle function. Pharmacol Rev, 1996; 48: 531-565.
22. Elorriaga M, Anselmi E, Hernandez JM, Docon P, Ivora D; The source of Ca^{2+} for muscarinic receptor-induced contraction in rat ileum. J pharm Pharmacol., 1996; 48: 817-819.
23. Zhang WW, Li Y, Wang XQ, Tian F, Cao H, Wang MW, Sun QS; Effects of magnolol and honokiol derived from traditional Chinese herbal remedies on gastrointestinal movement. World J Gastroenterol, 2005; 11(28): 4414-4418.
24. Brown JH, Taylor P; Cholinergic agonists. In: Brunton I.I., Lazo J.S., Parker K.I. (Eds), The pharmacological Basis of therapeutics., 11th ed. McGraw-Hill, New York, USA; 2006.
25. Rahwan RG, Faust MM, Witiak DT; Pharmacological evaluation of new calcium antagonists: 2-substituted 3-dimethylamino-5,6 methylenedioxyindenes. Pharmacol Exp Ther., 1977; 201: 126-137.
26. El Bardai S, Hamaide MC, Lyoussi B, Quetin-Leclereq J, Morel N, Wiborn M; Marrubenol interacts with the phenylalkylamine binding site of L-type calcium channel. Eur J pharmacol., 2004; 492 : 269-272.
27. Gilani AH, Aziz N, Khurram IM, Chaudhary KS, Iqbal A; Bronchodilator, spasmolytic and calcium activities of *Nigella. sativa* seeds (Kalonji), a traditional herbal product with multiple medicinal uses. J Pak Med Assoc., 2001; 51: 115-120.
28. Márquez AC, Lara OF, Esquivel RB, Mata ER, Luna A; Plantas Medicinales de Mexico II. Composición, usos y actividad biológica, UNAM, Mexico; 1999.
29. Gebhardt Y, Witte S, Forkmann G, Lukacin R, Matern U, Martens S**;** Molecular evolution of flavonoid dioxygenases in the family Apiceae. Phytochemistry, 2005; 66: 1273-1284.
30. Moehle B, Heller W, Wellmann E; UV-induced biosynthesis of quercetin 3-O-beta-D-glucuronide in dill *Anethum graveolens* cell cultures. Phytochemistry, 1985; 24: 465-468.