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SPASMOLYTIC ACTIVITY OF FLAVONOID EXTRACTS FROM SOME MEDICINAL PLANTS USED AS ANTIDIARRHEAL AGENTS IN TRADITIONAL MEDICINE IN KINSHASA- DRCONGO

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ABSTRACT

Seventeen flavonoid extracts from some medicinal plants used as antidiarrheal agents were evaluated for their potential spasmolytic activity at concentrations from 10^{-1} to 6.10^{-1} mg/ml in organ bath against acetylcholine (ACh) and depolarizing solution rich in KCl (DSR-KCl)-induced contrations on isolated guinea-pig ileum. Resulted indicated that 8 (47.05%) flavonoid extracts inhibited ACh and DSR KCl-induced contractions of isolated guinea-pig ileum more than 60%. The most active samples produced more than 65% and more than 60% inhibition of ACh and DSR KCl-induced contractions of isolated guinea-pig ileum respectively at a tested concentration from $1.6.10^{-1}$ to 2.10^{-1} mg/ml. They included flavonoid extracts from *Alcornea cordifolia* leaves (73.90 ± 0.06 and 68.36 ±0.03 %), *Ceiba pentandra* stem bark (66.30±0.02 and 63.25±0.05%), *Euphorbia hirta* whole plant (75.52 ± 0.01 and 72.15 ± 0.05%), *Garcinia kola* stem bark (75.20 ± 0.02 and 66.35 0.10 %), *Harugana madagascariensis* stem

bark (66.00±0.10 and 55.21±0.13), *Morinda morindoides* leaves (78.81 ± 0.01 and 75.65 ± 0.03%), *Psidium guajava* (71.75 ± 0.04 and 68.54 ± 0.02 %) and *Tithonia diversifolia* leaves. (71.25±0.06 and 67.25±0.03%). A group of 5 (29.41%) flavonoid extracts including those from *Cassia siamea*, and *Pentaclethra macrophylla* stem bark, *Jatrpha curcas, Nauclea latifolia* and *Phytolaca dodecandra* leaves inhibited ACh and DSR KCl-induced contractions on guinea-pig ileum with more than 50% but less than 60% at tested concentration of 2.10^{-1} mg/ml. 3 (17.64%) flavonoid extracts from *Bridelia ferruginea* and *Crossopterix febrifuga* leaves, an *Hymenocardia acida* stem bark produced more than 50% inhibition of ACh and less than 50% inhibition against DSR KCl-induced contractions on isolated organ. The 1 (5.88%) flavonoid extract from *Mangifera indica* stem bark produced less than 50% inhibition of contractions induced by both agonists on the isolated organ (16-30% inhibition). These reported results demonstrated that more selected flavonoid extracts possess interesting spasmolytic activity which can be partly considered as responsible of the spasmolytic activity of these medicinal plant extracts and explain their traditional use for the treatment of diarrhea in traditional medicine in Kinshasa-Democratic Republic of Congo and other African countries.

KEYWORDS: Medicinal plants, flavonoid extracts, spasmolytic activity, diarrhea.

INTRODUCTION

Irritable bowel syndrome (IBS) is assimilated to functional gastrointestinal disorders and its cause is until now unknown while its symptoms comprise diarrhoea, constipation and abdominal pains and various cramps.[1] Its treatment need the use of synthetic medicines including antispasmodic drugs.[2]

Antispasmodic drugs are composed with two important groups: one is antimuscarinic or anticholinergic agents such many flavonoids and its derivatives from different medicinal plants and another is calcium blockers.[3] Sometimes, the use of these kind of drugs causes unwanted side effects such as urinary retention, dry mouth, irritation, headache, nausea, vomiting and constipation recognized to antimuscarinic and calcium blocker drugs according to the case.[4]

In developing countries, morbidity and mortality of childhood caused by diarrhoea and other gastrointestinal problems are major health preoccupation since it is frequently estimated 10 million deaths in children under 5 years.[5] In these countries, people are still relying to

traditional medicine using different aqueous preparations based on various medicinal plants claimed by traditional healers to cure the disease in their daily practices. In the present study, all selected medicinal plants are used in Kinshasa-Democratic of Congo to treat diarrhoea and were scientifically investigated to prove their antidiarrheal activity in different experiment models such as by evaluation their antispasmodic, antiamoebic and antibacterial activities and many of them have given positive responses in all biological tests[6-8], but active fractions contening a specific chemical group responsible of evaluated activities were not reported. Recently,[9] have only reported the active soluble fractions for the antibacterial activity of these selected medicinal plants including fractions rich in alkaloids, flavonoids, steroids and terepenes, and saponins found to exhibit antibacterial on a large game of bacteria implicated in diarrhea. Thus, the present study was conducted to evaluate antispasmodic activity of flavonoid extracts from these selected different medicinal plants by reporting their effective doses 50 (ED_{50}).

2. MATERIALS AND METHODS

2.1. Plant materials: All plant materials were collected in Kinshasa (DRCongo) and plants were identified at National Institute of Studies and Research in Agronomy (NISRA), Department of Biology, Faculty of Sciences, University of Kinshasa. A voucher specimen of each plant was deposited in the herbarium of this institute. All plant materials were dried at room temperature and reduced to powder by using an electronic blender.

2.2. Preparation of aqueous extracts and extraction of flavonoids

Aqueous extracts were prepared by heating 50 g of each plant material in 200 ml distilled water for 15 minutes on a hotplate. After cooling and filtration on a paper filter Watman N° 1, each filtrate was evaporated under reduced pressure by rotary evaporator to give corresponding dried extract. On the other hand, for the extraction of flavonoids, 10 g of each dried extract were dissolved in 100 ml distilled water and filtered. Each filtrate was acidified with HCl 0.02 N and exhaustively extracted with isoamylic alcohol. The alcoholic fractions was evaporated under reduced pressure by rotary evaporator yielding corresponding dried flavonoid extracts responding positively to Shinoda's reagent (HCl + magnesium) and Neu's reagent (diphenylboric ethanolamine complex acid).[10,11]

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: KCl: 2.2, $MgCl_2$: 0.11, $NaH_2PO_4.2H_2O$: 0.42, $CaCl_2$:1.8, NaCl:137, $NaHCO_3$:11, glucose:5.6) or depolarizing solution rich in KCl (mM: NaCl: 2.7, KCl:100, $NaHCO_3$:15, $CaCl_2$:1.25, $MgCl_2$:12.5, glucose:11) gassed with 95% O_2 and 5% CO_2.

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 5.10^{-7} M acetylcholine (ACh) or depolarizing solution rich in KCl (DSR KCl) to have tree equivalent contractions and was after plentifully washed with Tyrode's solution to eliminate the presence of agonists in organ bath. To evaluate antispasmodic activity, 2 ml of agonists were removed in organ bath and replace by 2 ml of tested samples (2.10^{-3} - 10^{-1} mg/ml in organ bath) and left in contact with isolated guinea-pig ileum for 15 minutes.

The effects of flavonoid extracts of 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:

$$\% \text{ Inhibition} = \frac{Cag - Cts}{Cag} \times 100$$

Where Cag is the amplitude level of the contractions (cm) induced by agonists and Cts is the amplitude level of the contractions (cm) induced by tested samples. Effective doses 50 (ED_{50}) were derived from linear curves doses-responses.[8,12]

2.4. Statistical analysis 7: 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

Gastrointestinal motility is regulated by various mediators especially ACh and DSR KCl. Contractions induced by both agonists on isolated guinea-pig ileum encourage influx of extra-cellular calcium and stimulate the realising of calcium from sarcoplasmic reticulum [3]. In process of ileum relaxation, the relaxant mechanism is stimulated by antagonists of both

agonists used.[14] It is well known that isolated guinea-pig ileum is mainly used in experiments that study effects of samples from medicinal plant extracts and their fractions on gastrointestinal movement due to their capacity to inhibit ACh or DSR KCl-induced contractions on this organ. Flavonoids are known to relax pre-contracted intestinal smooth muscle and to delay intestinal transit (William et al., 2015) and exerted spasmolytic activity.[15-17]

The spasmolytic activity was analysed for all flavonoid extracts of and showed their overall inhibitory effects on ACh and DSR KCl-induced contractions of isolated guinea-pig ileum. They significantly inhibited the maximum ileum contractions at the contractions from 10^{-1} to 6.10^{-1} mg/ml presenting the amplitude contractions decrease ranging from 2.11 to 78.8% against ACh and 3.20 to 74.65% against DRS KCl with significant difference ($p < 0.05$) between them (Table 1). All extracts of flavonoids caused a concentration-dependent inhibition of spontaneous contractions induced by both agonists on isolated guinea-pig ileum, thus showing spasmolytic activity with different magnitudes ($p < 0.05$).

To assess whether the spasmolytic activity of these flavonoids extracts were also mediated through Ca^{2+} channel blockade, high concentration of K^+ (100 mM) was used to depolarize the preparation.[18] Moreover, a used high K^+ concentration > 30 mM, is known to produce smooth muscle contractions through the opening of voltage-dependent L-type Ca^{2+} channels, thus allowing influx of extra-cellular Ca^{2+} producing contractile action[19] and any substance causing inhibition of the high K^+ induced contractions is considered as an inhibitor of the Ca^{2+} influx[20] or a Ca^{2+} influx blocker as explained by.[19] Calcium antagonists are considered as an important therapeutic group and are characterized by their dose-dependent inhibition of slow entry of this cation.[21] Thus, the spasmolytic effect of these flavonoid extracts, as evident by the relaxation of high K^+-induced concentrations may be due to the channel blockade as also reported by[20] for the fractions and extracts of *Euphorbia granualta*, and mediated possibly through Ca^{2+} antagonist effects which can explain their therapeutic usefulness in hyperactive gut disorders, such as abdominal coli and diarrhea as these selected medicinal plants are known to be useful in such disorders. Many extracts of flavonoids in the present study caused the relaxation of high K^+ in a dose-dependent manner, indicated the involvement of Ca^{2+} channel blocking activity in spasmolytic activity because substance inhibited high K^+-induced contractions is denoted CCB (calcium channel blocker).[22] This observed calcium channel blocking effect is due to the presence of flavonoids, as evident

from phytochemical screening, because these constituents have been reported previously to have calcium channel blocking.[23]

Results presented in Table 1 revealed that extracts of flavonoids from *Alcornea cordifolia*, *Morinda morinodie*s and *Psidium guajava* leaves produced more than 70 and 68% inhibition of contractions induced by ACh and DSR KCl respectively on isolated guinea-pig ileum at a tested concentration of 2.10^{-1} mg/kg in organ bath. These flavonoid extracts exerted spasmolytic activity with effective dose 50 (ED_{50}) ranging from 0.70.10- to $1.87.10^{-1}$ mg/ml

Table. 1. Spasmolytic activity of flavonoid extracts from selected medicinal plants.

Plant species	Used parts	Concentration (mg/ml)	% IACh	% IDSR KCl
Alchornea cordifolia Mull-Arg.	L	2.10^{-2}	5.70±0.02	3.20±0.04
Euphorbiaceae		$1.2.10^{-1}$	26.00±0.05	17.00±0.08
		$1.6.10^{-1}$	73.90±0.06	68.36±0.03
Bridelia ferruginea Benth.	L	2.10^{-1}	16.00±0.00	11.03±0.02
Euphorbiaceae		2.10^{-2}	24.00±0.07	18.36±0.05
		2.10^{-1}	52.12±0.02	37.85±0.04
Cassia siamea Lam.	Sb	2.10^{-2}	24.56±0.03	18.56±0.05
Caesalpiniaceae		2.10^{-1}	52.12±0.12	45.25±0.05
		10^{-1}	55.24±0.04	52.63±0.02
Ceiba pentandra (L.) Gaertn	Sb	2.10^{-2}	0.00±0.00	0.00±0.00
Bombaceae		2.10^{-2}	32.00±0.01	27.12±0.04
		2.10^{-1}	66.70±0.02	63.25±0.05
		2.10^{-1}	52.12±0.04	40.23±0.02
Crossopterix febrifuga Benth	L	2.10^{-2}	37.40±0.00	26.54±0.04
Rubuace*ae*		2.10^{-1}	55.56±0.06	44.68±0.04
		10^{-1}	42.32±0.07	36.55±0.03
Euphorbia hirta L.	Wp	2.10^{-1}	75.56±0.08	72.65±0.05
Euphorbiaceae		2.10-1	55.23±0.03	52.30±0.05
		10^{-2}	48.69±0.02	44.63±0.04
Garcinia kola Heckel	Sb	2.10^{-2}	18.50±0.01	15.30±0.11
Clusiaceae		$1.2.10^{-1}$	51.80±0.13	46.25±0.09
		2.10^{-1}	75.20±0.01	66.35±0.10
Harugana madagascariensis	Sb	2.10^{-2}	12.50±0.01	8.65±0.04
Lam ex Poir Hypericaeae		2.10-1	55.87±0.02	52.69±0.04
		10^{-1}	40.00±0.03	36.25±0.11
		2.10^{-1}	66.00±0.10	55.21±0.13
Hymenocardia acida Tull	Sb	2.10^{-2}	2.91±0.07	1.02±0.02
Euphorbiaceae		2.10^{-1}	55.81±0.04	44.12±0.06
		10-1	42.35±0.02	37.56±0.07
Morinda moridoides (Baker)	L	2.10^{-2}	48.56±0.01	45.68±0.08
Milne-Readh Rubiaceae		2.10-1	74.21±0.02	71.35±0.04
		10-1	65.62±0.05	63.56±0.04
Jatropha curcas L.	L	2.10^{-2}	44.25 ±0.01	40.06±0.03
Euphorbiaceae		2.10-1	53.21±0.06	51.25±0.05

		10-1	48.24±0.02	43.56±0.11
		$1.2.10^{-1}$	58.23±0.04	51.24±0.02
		2.10^{-1}	78.81±0.01	74.65±0.03
Mangifera indica L.	Sb	2.10^{-2}	0.00.±0.00	0.00±0.00
Anacardiaceae		2.10^{-1}	25.65±0.12	16.52±0.09
		10^{-1}	11.12±0.04	8.65±0.02
Nauclea latifolia Smith	L	2.10^{-2}	42.06±0.02	39.60±0.05
Rubiaceae		2.10-1	56.24±0.12	51.24±0.03
		10-1	48.35±0.11	45.65±0.07
		2.10^{-1}	11.12±0.02	7.32±0.08
Pentacletra macrophylla Benth. Mimosaceae	Sb	2.10^{-2} 2.10^{-1} 10-1	32.05±0.05 53.65±0.04 48.56±0.02	28.56±0.05 51.25±0.06 44.06±0.03
Phytolacca dodecandra L'Herit. Phytolacaceae	L	2.10^{-2} 2.10^{-1}	35.15±0.10 54.03±0.04	30..06±0.09 50.25±0.02
		10^{-1}	46.25±0.11	44.65±0.02
		2.10^{-1}	58.82±0.12	53.65±0.10
Psidium guajava L.	L	2.10^{-1}	72.25±0.04	68.54±0.02
Myrtaceae		1.10^{-1}	61.32±0.05	57.56±0.01
Thitonia diversifolia (Hasmel) A. Gray Asteraceae	L	10^{-1} 2.10-2 2.10-1 10-1	57.62±0.07 49.50±0.05 71.25±0.06 55.68±0.02	52.36±0.02 46.21±0.08 67.25±0.08 52.36±0.07
Atropine sulfate		10	100.00±0.00	0.00±0.00
Papaverine chlorhydrate		10	100.00±0.00	98.94±0.34

L: leaves, Sb: stem bark, % IACh: % inhibition of acetylcholine, % IDSR KCl: % inhibition of depolarizing solution rich in KCl.

Suggesting their much potential effect on inhibition contractions induced by both agonists. Their activities were higher ($p < 0.05$) compared to other selected flavonoids extracts and showed significant difference ($p < 0.05$). Flavonoid extracts from *Bridelia ferruginea*, *Pentachletra macrophylla* stem bark and *Crossopterx febrifuga* leaves, caused more than 50%, but less than 60% inhibition of ACh-induced contractions on isolated guinea-pig ileum and more than 35% inhibitions of contractions of the isolated organ induced by DSR KCl at the tested concentration of 2.10^{-1} mg/ml (Table 1). The remaining flavonoid extracts from other selected plant produced an inhibition of contractions induced by both agonists less than 50%, but significant at the same tested concentration. Specially, flavonoid extract from *Ceiba pentandra* inhibited contractions of isolated guinea-pig ileum induced by both agonists at less percentage than 25% at a tested concentration of 6.10^{-1} mg/ml (Table 2) while that from *M. indica* stem bark produced 25.65±0.02 and 16.52±0.09% inhibition of ACh and DSR KCl-induced contractions of the isolated organ respectively at a tested concentration of 2.10^{-1} mg/ml suggesting its ED_{50} greater than 2.10^{-1} mg/ml for both agonists.

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 [24]. (Table 2). Periodic depolarization and repolarization of the tissues due to the influx of calcium into sarcoplasmic reticulum trough voltage-dependent calcium.

Table. 2: Effective doses 50 (ED_{50}) of flavonoid extracts.

Extracts of flavonoids	Ach (ED_{50}, mg/ml	DSR KCl (ED_{50} mg/ml)
A. cordifolia	$1.40.10^{-1}$	$> 2.10^{-1}$
B. ferruginea	$1.87.10^{-1}$	$> 2.10^{-1}$
C. siamea	$1.17.10^{-1}$	$1.25.10^{-1}$
C. pentandra	$1.11.10^{-1}$	$> 2.10^{-1}$
C. febrifuga	$1.97.10^{-1}$	$> 2.10^{-1}$
E. hirta	$1.02.10^{-1}$	$1.12.10^{-1}$
G. kola	$1.14.10^{-1}$	$1.24.10^{-1}$
H. madagascariensis	$1.37.10^{-1}$	$1.47.10^{-1}$
H. acida	$1.82.10^{-1}$	$> 2.10^{-1}$
J. curcas	$1.75.10^{-1}$	$1.93.10^{-1}$
M. morindoides	$0.70.10^{-1}$	$1.05.10^{-1}$
M. indica	$> 2.10^{-1}$	$> 2.10^{-1}$
N. latifolia	$1.82.10^{-1}$	$1.95.10^{-1}$
P. macrophylla	$1.50.10^{-1}$	$1.82.10^{-1}$
P. dodecandra	$1.91.10^{-1}$	$1.97.10^{-1}$
P. guajava	$1.68.10^{-1}$	$1.85.10^{-1}$
T. diversifolia	$1.54.10^{-1}$	$1.64.10^{-1}$
Atropine Papaverine	$0.03.10^{-1}$ $0.05.10^{-1}$	0.00±0.00 $0.03.10^{-1}$

ED_{50}: effective doses 50.

Channel are known as events responsible for spontaneous intestinal responses.[25]

According to the above observation, it has been suggested that substances that inhibit KCl-induced contractions act via blocking the channels. The most active sample was extract of flavonoids from *M. morindoides* leaves with ED_{50} of $0.70.10^{-1}$ mg/ml followed by that *E. hirta* whole plant with ED_{50} =$1.02.10^{-1}$ mg/ml and *G. kola* stem bark with ED_{50} = $1.14.10^{-1}$ mg/ml against ACh-induced contractions of isolated guinea-pig ileum while the remaining flavonoid extracts exhibited good spasmolytic activity with ED_{50} values ranging from $1.30.10^{-1}$ to $1.87.10^{-1}$ mg/ml showing significant difference of effect ($p < 0.05$) compared between them (Table 2). Against DRS KCl-inducted contractions of isolated guinea-pig ileum, it was observed that flavonoid extracts from *C. siamea*, *C. pentandra*, *G. kola* and *H. madagascariensis* stem bark, *E. hirta* whole plant, *M. morindoides* and *P. guajava* leaves, *G. kola* and *P. macrophylla* stem bark and leaves exhibited good spasmolytic activity with

E_{50} values ranging from $0.70.10^{-1}$ to $1.47.10^{-1}$ mg/ml. The same level of activity against this last agonist was obtained with flavonoid extracts from *J. curcas*, *N. latifolia* and *T. diversifolia* leaves and *P. macrophylla* stem bark with ED_{50} values ranging from $1.64.10^{-1}$ to $1.97.10^{-1}$ mg/ml. The remaining flavonoid extracts had $ED_{50} > 2.10^{-1}$ ml/ml (Table 2) and were considered as less active. For these effects against both agonists, extract of flavonoids from *M. morindoides* leaves showed higher activity ($p < 0.05$) (Table 2) followed by, *E. hirta* whole plant, *C. siamea*, *H. magadagscariensis* and *G. kola* stem with $ED_{50} < 1.50.10^{-1}$ mg/ml.

In addition the spasmolytic effects of these flavonoid 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 flavonoid extracts provided also the sound justification of their antidiarrheal properties.

Moreover, aglycone flavonoids were previuosly reported to inhibit intestinal motility and secretion[26] confirming their antidiadiarrheal activity in addition to their spasmolytic effect as mentioned below. 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 flavonoids extract have a papaverine-like effect.

Beside flavonoids reported to exhibit antispasmodic activity[13,16,27-31], other secondary metabolites such as alkaloids[32-40], saponins[22,40], steroids and terpenoids[22,41], and tannins [42] are reported to exhibit spasmolytic activity in different experimental models. Papaverine chlorhydrate tested at 40 µg/ml in organ bath produced 100% inhibition of ACh and DSR KCl while atropine sulphate produced 100% inhibition only against ACh and was devoid with effect against DRS KCl-induced contraction of isolated organ. Although the obtained data from this study demonstrated spasmolytic property of these selected flavonoid extracts, their efficiencies still lower compared to atropine and papaverine used as spasmolytic reference products.

4. CONCLUSION

In summary, the present findings showed that all selected extracts of flavonoids were tested in aglycone forms from these selected medicinal plants used to treat diarrhea and exhibited interesting spasmolytic activity mediated possibly through blockade of calcium channels as well as calcium from intracellular stores. They can be partly considered as responsible of the antispasmodic activity of aqueous extracts of these selected medicinal plants previously reported by [6-8]. This evaluated activity for flavonoid extracts of these selected medicinal more demonstrate and confirm their antispasmolytic activity previously reported as mentioned above. The reported results can also partly support and justify the traditional use of these selected medicinal plants in traditional medicine in Kinshasa-Democratic Republic of Congo (DRCongo) and other African countries for the treatment of diarrhea.

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