The Journal of Phytopharmacology 2022; 11(3):159-163
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2022; 11(3): 159-163
May- June
Received: 16-03-2022
Accepted: 13-05-2022

doi: 10.31254/phyto.2022.11305

Wensleslace Landry Mve Mendame
Laboratoire de Physiologie Animale : Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon

Boris Achille Eyi Mintsa
Laboratoire de Physiologie Animale: Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon

Alban-Michel Nguema Nguema
Laboratoire de Physiologie Animale : Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon

Arnaud Brice Pambo-Pambo
Laboratoire de Physiologie Animale : Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon

Ibrahim
Laboratoire de Physiologie Animale: Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon

Correspondence:
Dr. Wensleslace Landry Mve Mendame
Laboratoire de Physiologie Animale: Electrophysiologie-Pharmacologie, Unité de Recherche Agrobiologie, Université des Sciences et Techniques de Masuku (USTM), BP 943 Franceville, Gabon
Email: landry201414@gmail.com

Phytochemical screening and effects of aqueous extract of *Acalypha wilkesiana* Müll. Arg on isolated toad heart

Wensleslace Landry Mve Mendame, Boris Achille Eyi Mintsa, Alban-Michel Nguema Nguema, Arnaud Brice Pambo-Pambo, Ibrahim

ABSTRACT

Medicinal plants are real sources of bioactive molecules. The objective of the present study was to assess the effects of aqueous extract of Acalypha wilkesiana (ALWILEXT) on toad heart. Phytochemical screening of the aqueous extract revealed the presence of bioactives, namely: coumarins, flavones, polyphenols, alkaloids, tannins, terpenes and saponosides. The results obtained show that ALWILEXT, at concentrations ranging from 5.10-5 to 5.10-5g / ml, causes negative inotropic and chronotropic effects on the isolated toad heart, similar to those of acetylcholine although less marked. These effects suggest the presence of adrenomimetics in the aqueous extract. The aqueous extract of Acalypha wilkesiana is said to contain concomitantly adrenomimetics which would justify its use in the treatment of hypertension.

Keywords: *Acalypha Wilkesiana*, Phytochemical Screening, Inotropic, Chronotropic, Heart.

INTRODUCTION

The therapeutic use of the virtues of plants is an integral part of ancestral practices. Indeed, man has long used traditional herbal remedies without knowing what their beneficial actions were due to. To this day, plants are still a valuable source of food and remedies used by people in developing countries, especially people in Africa [1].

High blood pressure (hypertension) is one of the diseases that is steadily increasing worldwide. It represents the major risk of cardiovascular disease (CVD) as well as stroke [2]. A number of factors causes high blood pressure such as aging, sedentary lifestyle, harmful use of tobacco and alcohol but also poor diet [3]. The high cost of pharmaceuticals leads nearly 85% of people in developing countries to use herbal medicine [1]. The World Health Organization (WHO) estimates that in Africa, more than 80% of the population uses plants to meet their primary health needs [4]. This use of plants by humans is based on nutritional or therapeutic knowledge and their richness in secondary metabolites [5]. The effectiveness of plants in the treatment of high blood pressure is confirmed in numerous experimental studies [6,7].

Acalypha wilkesiana, a Euphorbiaceae widespread in West and Central Africa, is used as an ornamental plant but also exploited in the treatment of several pathologies. Indeed, this plant is used to treat fungal infections, diarrhea and diabetes [8,9]. The aim of the work is to determine the phytochemicals and to evaluate the pharmacological effects of the aqueous extract of *Acalypha wilkesiana* (ALWILEXT) on the contractile activity of the isolated heart of amphibian (toad).

MATERIALS AND METHODS

Plant material

The plant material consists of the leaves of *Acalypha wilkesiana*. Dr. IKABANGA Davy Ulrich of the Biology Department, where a sample of this harvested species is kept, identified these leaves.

Animal material

Toads of the *Bufo* (Batracian) genus whose mass varies between 60 and 70 g have been used for the study of the contractile activity of the heart. The animals were captured overnight at Université des Sciences et Techniques de Masuku (USTM) and then taken to the laboratory in the metal cages.

Preparation of *A. wilkesiana* extract

The leaves of *A. wilkesiana* harvested in the morning, were dried at room temperature for 3 weeks, away from the sun and then crushed using a Blender. Sixty grams (60g) of grind were mixed with 250 ml of each of the three selected solvents (water, ethanol and ethanol-water) for 24 hours under magnetic stirrer. The resulting macerates were filtered using cotton.

Phytochemical screening

Phytochemical characterizations of the extracts were carried out according to classical colorimetric techniques [10]. This qualitative study based on coloration and precipitation reactions by specific chemical reagents carried out on extracts of the leaves of *Acalypha wilkesiana*. The necessary extracts were obtained by extraction with the following solvents: ethanol and distilled water.

The purpose of these analyses is to highlight chemical groups with pharmacological properties.

Pharmacological substances

The substances used in this study: Locke Ringer Serum, Epinephrine and Acetylcholine. Epinephrine is an adrenergic receptor agonist. Acetylcholine is an antagonist of muscarinic receptors. Doses of *Acalypha wilkesiana* (ALWILEXT) were prepared from a physiological solution of Ringer.

Method of recording the mechanical activity of the isolated heart

The toad was decerebrate and demodulated, then fixed on a cork in the dorsal position by means of pins. The heart of the animal is laid bear with the help of scissors, skin, musculoskeletal wall and pericardium. The heart is quickly isolated as a result of a median thoracotomy. Once isolated, the heart is then attached to the infusion device. Its apex is connected to a recording stylus that transmits cardiac movements to a recording cylinder that drives a motor running at a constant speed (1 mm/s). The pharmacodynamic solutions used were dissolved in Ringer's physiological solution.

Statistical analyses

The results are expressed as the mean with a standard error (M±ESM). The INSTAT 3 software made it possible to carry out the statistical analysis of the data using the analysis variances (ANOVA) followed by the Dunnet t-test. The graphical representations were made using the GraphPad Prism 8.4.3 software (San Diego, CA USA).

RESULTS

Phytochemical screening

Phytochemical screening has made it possible to characterize the major groups of chemical families contained in the plant extract of *Acalypha wilkesiana*. *Table 1* presents the different chemical compounds in the leaf extract of *A. wilkesiana*.

Analysis of the results of phytochemical screening reveals the presence of many secondary metabolites. These results show an abundance of gallic tannins, catechuic tannins, polyphenols, flavonoids, flavones, flavanones, coumarins, alkaloids, anthracene compounds, reducing sugars, protozoans, gitonin's and gitoxigenin in aqueous and hydro-ethanolic extracts.

Flavanols, sterols and terpenes appear very weakly in these extracts. Saponosides are absent in all plant extracts. Regarding the alcoholic extract of the leaves of *A. wilkesiana*, polyphenols, sterols and terpenes are very present while flavanols, flavanones, flavones, reducing sugars, digitoxin and digitoxigenin do not appear in this extract (*Table 1*).

Table 1: Phytochemical screening of *Acalypha wilkesiana* Müll. Arg

Chemical compounds	Aqueous	Ethanol-water	Ethanol
Saponines	-	-	-
Tannins gallic	+++	++	+
Tannins catechic	+++	++	+
Total phenols	+++	+++	+++
Total flavonoids	+++	++	-
Flavonols	+	+	-
Flavones	++	++	-
Flavonones	+++	+	-
Coumarins	+++	+++	+
Alkaloids	+++	+++	++
Anthracenic compounds	+++	++	-
Sterols/Terpenes	+	++	+++
Reducing sugars	+++	+++	-
Proanthocyandins	+++	++	+
Gitoxin	+++	-	+
Gitoxigenin	+++	-	++
Digitoxin	-	++	-
Digitoxigenin	-	++	-

Very abundant: +++ ; Abundant : ++ ; Rare : + ; Absent : -

Effects of extract (ALWILEXT) and epinephrine on heart contraction

Figure 1 shows the record of the contractile activity of the heart as a function of concentrations. In this experiment, A. *wilkesiana* aqueous extract (ALWILEXT) causes a decrease in dose-dependent heart contractions. From 5.10-5mg/ml to 5.10-2mg/ml, the extract causes a decrease in contractions compared to the control and epinephrine ($p<0.05$; $p<0.001$). The threshold effect of ALWILEXT is 3.10-2mg/ml, the maximum decrease is observed at 5.10-2mg/ml.

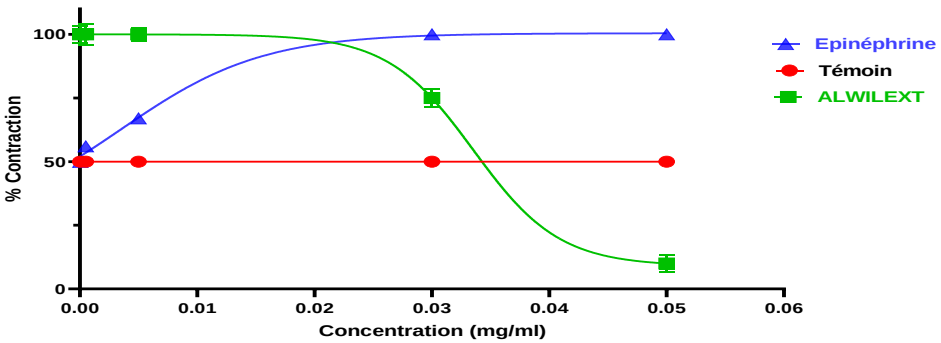

Figure 1: Effects of extract (ALWILEXT) and epinephrine on heart contraction

Effects of extract (ALWILEXT) and acetylcholine on heart contraction

The contraction recorded by an isolated organ system is shown in Figure 2. In general, ALWILEXT causes a decrease in heart contractions induced by epinephrine (5.10-2mg/ml). The threshold of effect is 2.10-2mg/ml and the maximum decrease is observed at 5.10-2 mg/ml. At concentrations 5.10-5 g/ml at 5.10-2 mg/ml, acetylcholine significantly induces inhibition of contractions compared to control and extract ($p<0.05$; $p<0.001$). However, at concentrations 5.10-3 g/ml, 5.10-4 g/ml and 5.10-5 g/ml, there is no significant difference between the extract and the control.

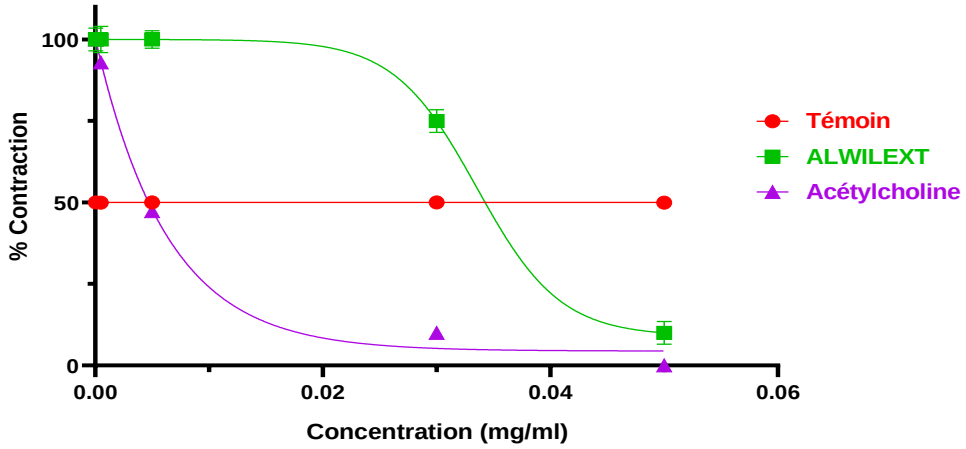

Figure 1: Evolution of contractions according to the extract and acetylcholine

Effects of extract (ALWILEXT), epinephrine and acetylcholine on heart rate

Figure 3 shows the changes in heart rate induced by pharmacological substances on the heart. Treatment with aqueous extract of *Acalypha wilkesiana* (ALWILEXT) induces a decrease in heart rate. At different concentrations from 5.10-5mg/ml to 5.10-2mg/ml, the aqueous extract induces a significant decrease in the frequency of contraction compared to the control ($p<0.001$). Regarding epinephrine, we observe an increase in contractions compared to the control and the extract. This dose-dependent increase in frequency compared to the extract is significant with a p-value of 0.001.

Acetylcholine significantly decreases heart contraction rates compared to epinephrine, extract and control at treated concentrations ($p<0.05$; $p<0.001$).

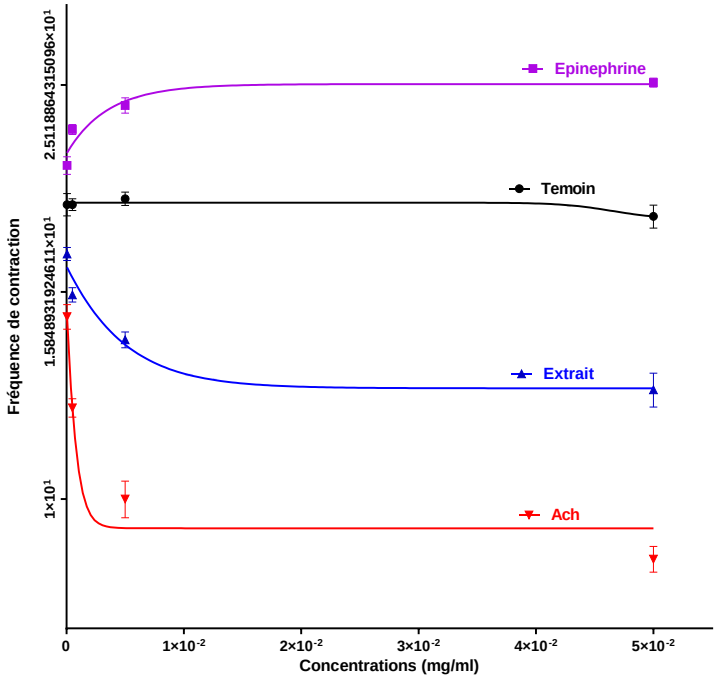

Figure 2: Variation in the frequency of contraction

Variation in the amplitude of contraction

The results of the study on interactions of extract and pharmacological substances on the mechanical activity of the isolated toad heart are summarized in Table 2. This table shows that at concentrations 5.10-3 and 5.10-2mg/ml, the aqueous extract induces decrease in the amplitude of contraction (negative inotropic effects) with a significant difference ($p<0.05$). Acetylcholine causes a decrease in the amplitude of contraction of the heart at all concentrations, this decrease is very significant ($p<0.001$).

Table 2: Variation in the amplitude of heart contraction

Concentrations (g/ml)	Amplitude de contractions					
	Control	Extract		Epinephrine		Acetylcholine	
		Amplitude (cm)	Variation (%)	Amplitude (cm)	Variation (%)	Amplitude (cm)	Variation (%)
5.10^{-5}	0,6 ± 0,0057	0,46 ± 0,036	23,34	0,69 ± 0,036	-15	0,56 ± 0,04	11,12
5.10^{-4}	0,61 ± 0,01	0,4 ± 0,015**	34,43	0,76 ± 0,02	-24,59	0,32 ± 0,026***	47,8
5.10^{-3}	0,613 ± 0,0057	0,31 ± 0,096***	49,43	0,8 ± 0,01	-30,5	0,22 ± 0,032***	63,94
5.10^{-2}	0,63 ± 0,0173	0,19 ± 0,04***	69,85	0,85 ± 0,03	-34,92	0,09 ± 0,05***	85

Values express percentage variations in amplitude of cardiac contacts compared to normal recordings (mean ± SD, n=3)
Control= Normal Ringer; ALWILEXT= Aqueous extract of *Acalypha wilkesiana*
Values express percentage changes in amplitude and frequency of cardiac contractions compared to normal recordings (mean ± SEM; n=3; *p<0.05; ***p<0.001).

DISCUSSION

The results of the phytochemical screening highlighted the presence of many secondary metabolites. Thus, in aqueous and hydro-ethanolic extracts (ethanol and water), chemical compounds such as gallic tannins, catechetical tannins, polyphenols, flavonoids, flavones, flavonones, coumarins, alkaloids, anthracenic compounds, reducing sugars, proanthocyanins, gitoxins and gitoxigenins appeared in abundant quantities. On the other hand, flavonols, sterols and terpenes were present in very small quantities.

As for the alcoholic extract of the leaves of *A. wilkesiana*, polyphenols, sterols and terpenes were present while flavonols, flavonones, flavones, reducing sugars, digitoxin and digitoxigenin were completely absent.

Secondary metabolites are indicated as molecules with the ability to provide plants with pharmacological properties such as antioxidant, antihypertensive properties. Indeed, some authors have shown that the flavonoids, alkaloids and polyphenols contained in *Helichrysium mechowianum* extract have an effect on the activity of the uterine muscle [10]. In addition, studies conducted by many authors have shown that flavonoids, tannins, polyphenols play a role in protection against platelet aggregations in the wall of blood vessels involving antihypertensive and vasorelasting properties [8-11]. Also, polyphenols, flavonoids and tannins are known to have relaxing effects on the heart muscle and alkaloids would induce a reduction in blood pressure. The richness of the aqueous extract of *Acalypha wilkesiana* in active chemical compounds would explain the use of this plant in traditional medicine to cure many diseases.

Pharmacological tests on the isolated heart of batracian show that, aqueous extract of *Acalypha wilkesiana* (ALWILEXT) causes negative inotropic effects between 5.10-5 and 5.10-2mg/ml and negative chronotropic effects between 5.10-2 and 10-2 mg/ml. Also, at concentrations 5.10-4 and 5.10-2mg / ml ALWILEXT leads to a decrease in frequency and a decrease in the amplitude of contraction between 5.10-4 and 5.10-2mg/ml on the isolated heart of batracian. This decrease in amplitude and frequency is comparable to that of acetylcholine, a reference molecule. These results suggest that ALWILEXT would act by binding to muscarinic cholinergic receptors.

Indeed, the chemical compounds contained in the extract of ALWILEXT would induce the chronotropic effect by binding on the muscarinic M2 receptors inducing the opening of potassium channels linked to the G proteins resulting in hyperpolarization of cells. As for the inotropic effect, it would be due to the decrease in the entry of Ca2+ into the cell leading to the inhibition of adenylcyclase.

These effects are similar to those reported on a large number of cardioactive medicinal plants [6,7]. The negative inotropic and chronotropic effects by the aqueous extract would indicate the presence of muscarinic cholinomimetic substances in this plant extract. Since the cholinergic receptors of the heart are muscarinic receptors, ALWILEXT compounds induce negative chronotropic and inotropic effects by hyperpolarization of nodal cells due to increased potassium [12,13].

These results deserve to be further developed using more elaborate pharmacological models while continuing to study the mechanism of action and signaling pathway of *Acalypha wilkesiana* extract.

CONCLUSION

At the end of this work, it appears that the aqueous extract of *Acalypha wilkesiana* is rich in coumarins, flavones, polyphenols, alkaloids, tannins, terpenes and saponosides. On the heart of the amphibian, ALWILEXT causes a decrease in the frequency and amplitude of contraction. These results justify the use of *Acalypha wilkesiana* in the treatment of hypertension in traditional medicine.

Acknowledgements

The authors would like to thank Mr. MENDAME NDONG Cyriaque for guiding them throughout the investigation.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. OMS. 2013. Aide-mémoire http://www.who.int/mediacentre/factsheets/fs355/fr/ , N°355. Statistiques Sanitaires Mondiales.
2. Rigaud S, Hanon O, Bouchacourt P, Forette F. Les complications cérébrales de l'hypertension artérielle chez le sujet âgé. Rev Med Interne. 2001;22:959-68.
3. Etou Ossibi AW, Dimo T, Elion-Itou RDG, Nsondé-Ntandou GF, Nzonzi J, Bilanda DC, *et al*. Effets de l'extrait aqueux de Lippia multiflora Moldenke sur l'hypertension artérielle induite par le DOCA–sel chez le rat. Phytothérapie. 2012;5:193-99.
4. OMS. Stratégie de l'OMS pour la médecine traditionnelle pour 2002-2005.WHO/EDM/TRM, Genève. 2002;p.65.
5. Nsi Akoué G, Obame LC, Ondo JP, Brama I, Otogo N'Nang ES, Tapoyo SY, *et al*. Phytochemical composition and antiradical activity of Sakersia africana Hook. f. medicinal plant from Gabon Sakersia total. Int. J. Biomol. Biomed. 2013;3:1–8.
6. Zahoui OS, Nene-bi SA, Soro TY, et Traore F. Etude des effets pharmacologiques de l'extrait aqueux de Heliotropium indicum Linn. (Boraginaceae) sur le cœur isolé de rat et l'aorte isolée de cobaye. Int. J. Biol. Chem. Sci. 2010;4(5):1610-20.
7. Ibrahim B, Attéké NC, Mougengui S, Lepengue NA, Issembe AY, Akoué NG, *et al*. Antihypertensive potential of aqueous extract of Nephrolepis biserrata leaves on toad aorta. Med & aro plant. 2015;5:1.
8. Odoh UE, Ndubuokwu RI, Inya-Agha SI, Osadebe PO, Uzor PF, Ezejiofor M, *et al*. Antidiabetic activity and phytochemical screening of Acalypha wilkesiana (Euphorbiaceae) Mull Arg. roots in alloxan-induced diabetic rats. Sci Res Essays. 2014;9(7):204-12.

9. Aladejimokun AO, Daramola KM, Osabiya OJ, Arije OC. Comparative Study of Phytochemical Constituents and Antimicrobial Activities of Acalypha wilkesiana and Acalypha godseffiana Extracts. J Adv Microbiol. 2017;4(1):2456-16.
10. Eyi Mintsa BA, Ibrahim, Mabika Mabika R, Mve Mendame WL, Lepengue AN, Souza A, *et al*. Etude phytochimique et activites biologiques de deux plantes : Alchornea floribunda (Euphorbiaceae) et Bridelia ferruginea (Phyllanthaceae). Int. J. Adv. Res. 2019;7(3),331-45.
11. Morand C, Milenkovic D. Polyphénols et santé vasculaire: mise en évidence du rôle direct des polyphénols dans les effets bénéfiques des agrumes dans la protection vasculaire. Innovations Agronomiques. 2014;42:47-62.
12. Feletou M, Busse R, Edwards G, Fleming I, Weston AH, Vanhoute PM, *et al*. Dialogue entre cellules endothéliales et cellules musculaires lisses. Médecine/Sciences. 2003; 19: 1242-1250.
13. Aubin M.C.2009. Etude de la fonction vasculaire et du remodelage cardiaque avant l'établissement de l'obésité et de la dyslipidémie chez les rats femelles Sprague-Dawley recevant une diète riche en gras. Thèse de Pharmacologie, faculté de médecine, université de Montréal, p. 192.

HOW TO CITE THIS ARTICLE
Mendame WLM, Mintsa BAE, Nguema AMN, Arnaud Brice Pambo-Pambo1 Ibrahim ABPPE. Phytochemical screening and effects of aqueous extract of Acalypha wilkesiana Müll. Arg on isolated toad heart. J Phytopharmacol 2022; 11(3):159-163. doi: 10.31254/phyto.2022.11305