



P-ISSN: 2349-8528

E-ISSN: 2321-4902

IJCS 2019; 7(3): 104-108

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Received: 26-03-2019

Accepted: 11-04-2019

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# Chemical screening, negative chronotropic and positive inotropic effects of aqueous extract of *Alchornea cordifolia* (Schummach. & Thonn) Mull. Arg. (Euphorbiaceae) on uterine motricity

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## Abstract

The uterus is the essential organ in reproduction. Its dysfunction can be the origin of the difficulties of conception, the miscarriages and the threats of abortion. To contribute to the valorisation of uterotrophic plants used in Congolese traditional medicine, we performed a chemical screening by tube reaction and evaluated the effect of *Alchornea cordifolia* leaves extract on the guinea-pig uterus. The aqueous extract of the leaves of *Alchornea cordifolia* contains many flavonoids, cardiotonic heterosides, anthocyanins and saponoids; moderate steroids and terpenoids; and a few alkaloids. This extract administered at the doses of 400 and 800 mg/kg by vaginal route increased significantly the amplitude of uterine contractions (\*\*\*)  $p < 0.001$  and latency ( $p < 0.01$ ), and decreased the frequency ( $P < 0.01$ ) of uterine contractions. These negative chronotropic and positive inotropic effects are related to the presence of its secondary metabolites. These results are in harmony with the use of this plant in traditional medicine as emmenagogue and oxytocic.

**Keywords:** Traditional medicine, *Alchornea cordifolia*, Aqueous extract, Phytochemistry, Uterus, Moderator, Positive inotropic

## Introduction

Uterine contractions (UC) are the motor of labor and delivery. They provide both cervical dilatation and fetal progression to the genital tract, and are essential to the proper functioning of the labor (Cabrol D., 2003) [8]. Too frequent or too long contractions can threaten the healthy fetus (Christophe Sabine, 2012). According to the World Health Organization (WHO, 1995), almost 75% of neonatal deaths occur as a result of prematurity. Many antiabortive drugs are available in conventional medicine practice (Audra *et al.*, 1991) [1], but in view the difficult of access to these products due to their high cost and national bad distribution, a large part of the sub-Saharan African population turns to traditional medicine.

In the Republic of Congo, *Alchornea cordifolia* is used in various treatments: malaria, fever, tooth decay, dental caries, amoebiasis, hemorrhoids, hygiene intimate, headache, venereal disease, various inflammations and metritis or vaginitis. It is also used as emmenagogue and oxytocic (Bouquet, 1969 et 1972) [5, 6]. *Alchornea cordifolia* is also widely used in many other African countries (Keharo et Adam, 1975; Adjanaoum *et al.*, 1988; Tona *et al.*, 1998; 1999; Nkounkou Loumpanguou *et al.*, 2005) [12, 2, 16, 14]. This work aims to identify the various secondary metabolites of *Alchornea cordifolia* (Euphorbiaceae) and to elucidate its effects on the in vivo uterine motricity in the guinea pig.

## Plant material

The leaves of *Alchornea cordifolia* were collected in Makana village (Pool Department, Congo) under the supervision of a health practitioner who knew the village and this plant perfectly. The plant was identified by the botanists of Centre d'Etudes sur les Ressources Végétales (CERVE), Congo Brazzaville, where a voucher specimen with number P. SITA N° 2899 has been deposited. A sample of leaves of *Alchornea cordifolia* was dried at ambient temperature (25 °C) in the Laboratoire de Physiologie et Physiopathologie Animales, at the Faculté des Sciences et Techniques of the Marien NGOUABI University in Brazzaville-Congo. Immediately after drying, the dried material was reduced to powder.

## Animals

Fifteen (15) female guinea pigs, weighing between 300 and 500 g, aged about 3 months, were raised in the Laboratoire de Physiologie et Physiopathologie Animales of the Faculté des Sciences et Techniques under standard conditions ( $\pm 25^{\circ}\text{C}$ , 12 h cycle Of light/darkness). They were fed with standard food and have free access to water.

## Choice of the plant

A bibliographic survey made it possible to make a list of the uterotrophic plants from which a plant should be chosen after a favorable opinion of the traditional health practitioner. We collected the information on the family, genus, species, vernacular name, part of the plant used (leaves, roots, barks or stems), therapeutic indications, method of preparation (decoction, maceration, infusion), Chemistry, toxicology and pharmacology of uterotrophic plants. *Alchornea cordifolia*, which has been the most widely available, very used, best tolerated and safest plant, according to the traditional health practitioner, among the most used and least explored pharmacologically for its uterotrophic effects according to the literature, was retained for the study.

## Product preparation

The dried material was powdered using a mechanical grinder. Forty (40) g of the powder of the leaves of *Alchornea cordifolia* are placed in 400 ml of water and boiling for 30 minutes at  $100^{\circ}\text{C}$ . After cooling, the decoction is filtered. The filtrate obtained is concentrated at  $50^{\circ}\text{C}$  in incubator. The anesthetic solution is obtained by dissolving 1.5 g of ethyl carbamate in 10 ml of distilled water. The physiological solution of Mc Ewen is prepared by dissolving 4.3 g of NaCl; 0.21 g KCl; 0.14 g of  $\text{CaCl}_2$ ; 0.054 g of  $\text{NaH}_2\text{PO}_4$ ; 0.5 g of  $\text{NaHCO}_3$ ; 0.015 g of  $\text{MgCl}_2$  and 1 g of glucose, in 500 ml of distilled water. All products obtained are kept in a refrigerator at  $2^{\circ}\text{C}$  (Binimbi Massengo, 2013).

## Chemical screening

To identify the different chemical groups characteristic of this plant, a chemical screening was carried out. We used the classical phytochemical test by using the method of reaction in tube:

- **Alkaloids:** from 5 ml of aqueous extract, add 1 ml of 1N hydrochloric acid (HCl) and a few drops of the Mayer reagent. The test is positive when a yellow precipitate is formed.
- **Tannins:** from 5 ml of aqueous extract, add 1 ml of an aqueous solution of iron (III) chloride. The test is positive when there is a green coloration.
- **Anthocyanins:** from 5 ml of aqueous extract, add 5 ml of 10% sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and then 5 ml of 20% ammonia ( $\text{NH}_3$ ). The test is positive when the coloration is accentuated by acidification and then turn to purplish blue in a basic medium.
- **Free Flavonoids:** Introduce 5 ml of aqueous extract, 5 ml of hydrochloric acid solution, 1 ml of iso-amyl alcohol and some magnesium chips into a test tube. There will appear on the supernatant of iso-amyl alcohol a coloration:
  - Orange pink: presence of flavones;
  - Purple pink: presence of flavanones;
  - Red: presence of flavonols and flavanols.
- **Free anthraquinones:** from 2.5 ml of the aqueous extract, add 1 ml of 10% sodium hydroxide (NaOH). The test is positive when there is a red coloration.

- **Sterols and tri-terpenes:** dissolve the dry extract in 1 ml of acetic anhydride and add 1 ml of chloroform. Divide the solution between two test tubes, the first one serves as a control. Using a pipette, place 1 ml of sulfuric acid in the second tube. The test is positive when a brownish or violet red ring coloration is formed at the contact zone of the two liquids, the supernatant is colored in green or violet.
- **Saponosides:** in a series of ten test tubes successively distribute 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 ml of decoction. Adjust the volume of each tube to 10 ml with distilled water and shake each tube in a horizontal position for ten seconds. Let each tube stand for 15 minutes and then measure the level of the persistent foam (Tona *et al*, 1988) [16].

## Pharmacological study

### Selection of animals in oestrus

In the conscious animal, about 1.5 ml of distilled water is introduced into the vaginal cavity by means of a syringe. Approximately 0.5 ml was removed and then spread on a slide. The smear thus produced is dried in oven, followed by fixation in alcohol before being stained with GIEMSA according to Papanicolaou techniques (Yener., 2007) [18]. The observation of the cells is carried out under the GX 100 with optical microscope. These observations help to distinguish the cells in relation to the different phases of the cycle: di-oesters, pro-estrus, estrus and post-estrus in order to select the animals in period of estrus, the period of the cycle during which the uterus presents more abundant contractions. The period of estrus is characterized by the presence of keratinized cells isolated or grouped in a cupboard.

### Anesthesia of the guinea pig

To anesthetize the animals we used an intraperitoneal injection of urethane (ethyl carbamate) 15% at a dose of 1.5 g/kg (Dimo and Al., 2003) [10].

### Recording of contractions

The anesthetized animal being in the dorsal position, a lateral incision is made at the level of the lumbar region; this operation allows direct access to the uterine horn, avoiding the digestive tract. The thread is passed under uterine horn. This thread is connected to a writing pen, according to the technique described by Ruckebusch (1969), modified by Binimbi (2013) [4]. Spontaneous contractions are recorded on a rotating cylinder. The recording device is a kymograph of Ludwing, rotating at a constant speed of 0.1 mm/s. The recording is done 10 min after administration of the extract.

### Treatment of animals

The doses of 400 and 800 mg/kg of the aqueous extract of leaves of *Alchornea cordifolia* selected in this study have been tested in rats already, for other pharmacological activities (Yengozo, 2014). The products are deposited directly into the uterus vaginally. Four (4) groups of five (5) guinea pigs are each constituted. In the first group (control) the animals received 10 ml/kg of distilled water each; in the 2<sup>nd</sup> group (reference) each animal is treated with 0.005UI/100g of oxytocin. The animals of the 3<sup>rd</sup> and 4<sup>th</sup> groups received the doses of 400 and 800 mg/kg of body weight, respectively.

### Statistical analysis

The results obtained are expressed as mean  $\pm$  ES for  $n = 5$  per group, using Microsoft Excel Windows 10. The results

obtained in the test groups were compared to the control group using the Student test with a significance of  $p < 0,05$ ;  $p < 0,01$  and  $p < 0,001$ .

## Results

### Chemical Screening

The different metabolites of *Alchornea cordifolia* leaves are presented in Table I.

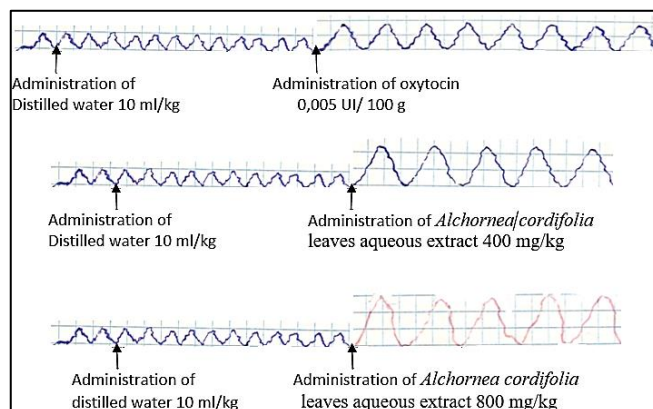
**Table I:** Different chemical groups of *Alchornea cordifolia* leaves aqueous extract

Secondary metabolites	<i>Alchornea cordifolia</i> extract
Alkaloids	+
Tannins	-
Anthocyanins	+++
Flavonoids	+++
Sterols and terpenoids	++
Saponoids	+++
Cardiotonic heterosids	+++
+ Presence ; ++ = Strong presence ; +++= Very strong presence ; - = absence	

### Pharmacological studies

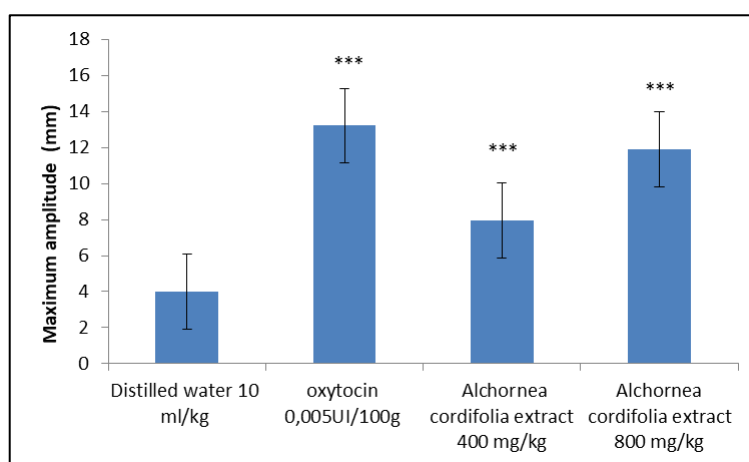
#### Uterotropic activity in female guinea pigs

The figure 1 shows the records of uterine contractions of animals treated with aqueous extract of *Alchornea cordifolia* at the doses of 400 and 800 mg/kg and the reference drug (oxytocin) at a dose of 0.005 IU/100 g on the female guinea pig's uterus. The amplitude of contractions increases with the dose but the number of contractions/minute decreases.

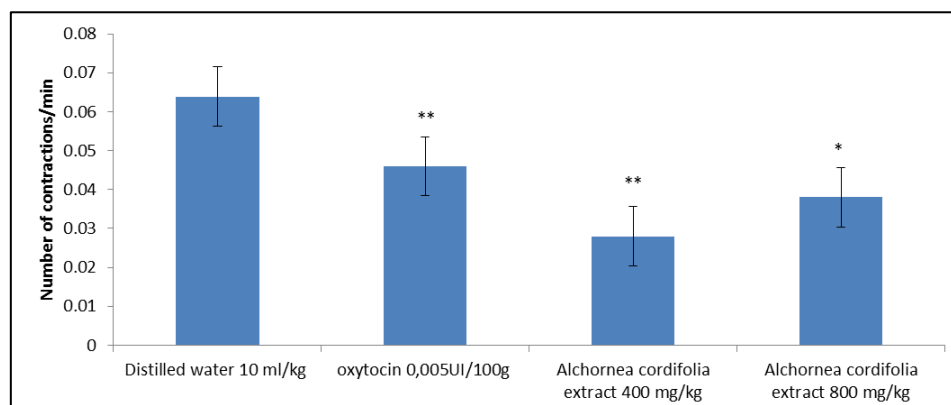


**Fig 1:** Typical records showing dose dependent inotropic positive and negative chronotropic effect of the aqueous extract of leaves of *Alchornea cordifolia* in uterus of guinea pigs

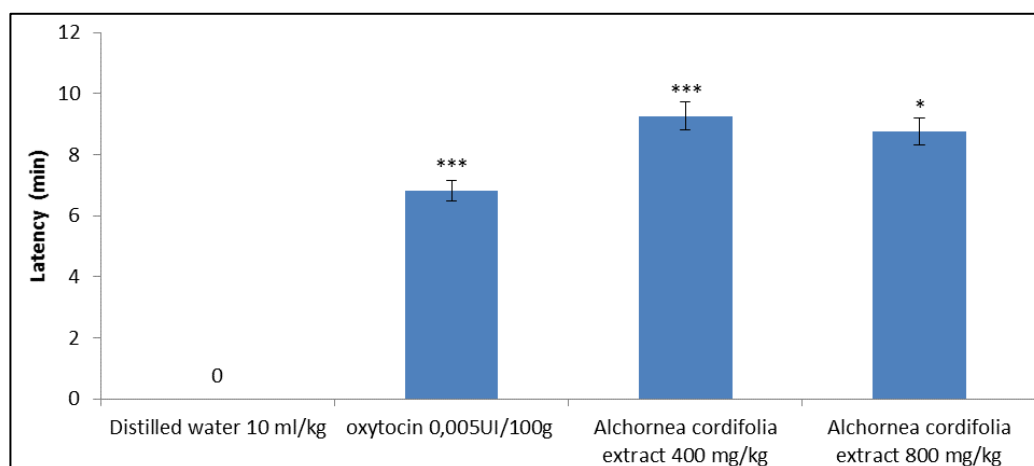
The aqueous extract of *Alchornea cordifolia* causes a dose-dependent and significant increase in amplitude of uterine contractions at 400 mg/kg ( $p < 0.001$ ) and 800 mg/kg ( $p < 0.001$ ) compared to the negative control (Figure 2). The aqueous extract of *Alchornea cordifolia* at the doses of 400 mg/kg ( $p < 0.01$ ) and 800 mg/kg ( $p < 0.05$ ) and oxytocin at a dose of 0.005 IU/100 g, increase significantly the rate of contraction of the guinea-pigs uterus (figure 3). The oxytocin, before its action has a latency time of 6.07 minutes. Aqueous extract of *Alchornea cordifolia* leaves at the doses of 400 mg/kg ( $p < 0.01$ ) and 800 mg/kg ( $p < 0.05$ ) also showed a significant decrease in latency time in dose dependent manner (figure 4).



**Fig 2:** Inotropic effect of *Alchornea cordifolia* leaves aqueous extract on the uterine motricity of the guinea-pigs. Results expressed as mean  $\pm$  ES,  $n = 5$ , \*\*\*  $p < 0.001$



**Fig 3:** Moderator effect of *Alchornea cordifolia* leaves aqueous extracts on the frequency of uterine contractions. The results are expressed as geometric mean  $\pm$  standard error,  $n = 5$ , \*\* $p < 0.01$ ; \* $p < 0.05$  compared with control.



**Fig 4:** Lifting effect of *Alchornea cordifolia* leaves aqueous extracts on the latent period of uterine motricity of female guinea pigs. The results are expressed as geometric mean  $\pm$  standard error,  $n = 5$ . \*\*\* $p < 0.001$  and \* $p < 0.05$  compared with control.

## Discussion

The chemical study aimed to identify the various secondary metabolites of *Alchornea cordifolia* (Euphorbiaceae). The results of the chemical screening of *Alchornea cordifolia* are different from previous work carried out by Bennet in 1950 by the absence of tannins in the leaves of *Alchornea cordifolia*. This can be due to the quality of the soil, the season of harvest or other various environmental factors. Our biological study aimed to assess the uterotrophic effect of *Alchornea cordifolia* leaves aqueous extracts on uterine motricity in female guinea pigs. This plant possesses interesting pharmacological properties, which have been studied in order to give scientific support to the reported therapeutic virtues cited by the traditional health practitioners. We have used the vaginal route for the administration of the extract because in traditional medicine, it is indicated with *Alchornea cordifolia* in hygiene intimate and in the gynecologic treatments in the form of bath and suppositories (Yengozo, 2014). The aqueous extract of the leaves of *Alchornea cordifolia* at the doses of 400 and 800 mg / kg and oxytocin at a dose of 0,005 U/100 g, increase the amplitude and the latency time, and decrease frequency of uterine contraction of female guinea-pigs. These results are similar with those found by Cabrol in 2003<sup>[8]</sup> with pharmacodynamic products. These positive inotropic and negative chronotropic dose dependent effects is in accordance with Massoma *et al.* (2014)<sup>[13]</sup> who have showed the contraceptive or antifertility effect of *Alchornea cordifolia* roots extract in female albino rats. Massoma *et al.* (2014)<sup>[13]</sup> showed a decrease in the rate of nidation and the progesterone level with the aqueous extract of *Alchornea cordifolia* roots. The aqueous extract could inhibit the production or the effect of progesterone on the endometrium (Franchin *et al.*, 2002)<sup>[11]</sup>. These effects may be due to the presence of sterols, terpenoids and alkaloids which are known for their effect on reproductive system in general and uterine activity in particular (Barjot *et al.*, 2001)<sup>[3]</sup>. These results are in harmony with the traditional use of *Alchornea cordifolia* as emmenagogue and oxytocic (Bouquet, 1969)<sup>[5]</sup>.

## Acknowledgments

The authors are indebted to the botanists of Laboratoire de Botanique du Centre d'Etudes sur les Ressources Végétales, Brazzaville-Congo for authenticating the plant material and the group of teacher researcher of Laboratoire de Physiologie et Physiopathologie Animales of Faculté des Sciences et Techniques, Marien Ngouabi University of Brazzaville for

their financial and technical support for the maintenance of animals. They particularly wish to thank the tradipractitioner who helped to select and collect the plant material.

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