

**Research Paper**

*Afr. J. Traditional,
Complementary and
Alternative Medicines*
www.africanethnomedicines.net

ISSN 0189-6016©2008**IN VIVO AND IN VITRO EFFECTS OF *BIDENS PILOSA L.* (ASTERACEAE) LEAF AQUEOUS AND ETHANOL EXTRACTS ON PRIMED-OESTROGENIZED RAT UTERINE MUSCLE.****Longo Frida^{a,d*}, Silvère Rakotonirina^b, Alice Rakotonirina^c and Jean-Pierre Savineau^d**

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Abstract

Bidens pilosa L. is an Asteraceae growing in tropical zones, and traditionally utilized worldwide in herbal medicine. The present work is based on its traditional use during child birth as a labour facilitator. *In vivo* tests of acute toxicity showed a weak toxic effect for both extracts but the toxicity of the ethanol extract ($LD_{50}=6.15\text{g/kg}$) was upper than that of the aqueous extract ($LD_{50}=12.30\text{g/kg}$). The three-days uterotrophic assay on immature mice showed body weight gain followed by a concentration-dependent decrease up to 4mg/g and a concentration dependent uterine wet weight increase. The ethanol extract exhibited the higher body weight gain representing $22.8\pm 0.7\%$, ($P\leq 0.001$), at the concentration of $500\mu\text{g/g/day}$, while the aqueous extract was significantly more efficient on the uterine wet weight gain of $0.24\pm 0.001\%$ ($P\leq 0.05$), at the concentration of $1000\mu\text{g/g/day}$. *In vitro* isometric contraction measurement of oestrogen-primed rat uterine strips showed a significant high aqueous extract-induced contractile effect from $0.03\text{-}1.97\text{mg/ml}$: on the amplitude of contraction ($EC_{50} = 0.44\pm 0.10\text{mg/ml}$, $P\leq 0.05$), and on the rate ($1.21\pm 0.25\text{mg/ml}$, $P>0.05$). In spite of the higher effect of the aqueous extract on the tonus ($57.23\pm 23\%$), the ethanol extract showed a high effect ($EC_{50}= 0.34\pm 0.09\text{mg/ml}$, $P\leq 0.05$). The weak toxicity, the estrogenic-like and the oxytocic-like activities observed could explain the empirical use of *Bidens pilosa* leaf aqueous extract as an uterotonic preparation to enhance labour, probably due to the presence of biologically active-compound(s) which act directly on the uterine muscle.

Key words: *Bidens pilosa L.* Leaf, Uterotrophic, Uterotonic, Mouse, Rat.

Introduction

Bidens pilosa L. is a perennial weed herb (Asteraceae) growing in tropical and subtropical regions all around the world and widely used. All parts of the plant contain bioactive molecules which react in curing several diseases in either association or not. Previous works have been done with regard to many of its biological properties such as anti-microbial (Andersen et al., 2006; Rojas et al., 2006), anti-inflammatory (Pereira et al., 1999), anti-malarial (Krettli et al., 2001; Andrade-Neto et al., 2004; Oliveira et al., 2004)

hypoglycaemic (Alarcon-Aguilar et al., 2002; Chang et al., 2004; Chang et al., 2005) and pesticidal actions (Gilbert et al., 1999; Machado et al., 2005).

In Cameroon, since the last 20 years, the framework of the laboratory of animal physiology of the Faculty of Sciences of the University of Yaounde I is to study modes of action of some medicinal plants currently used in rural zones by tradipracticitionners. Different *Bidens pilosa* leaf extracts were tested on various smooth muscles. Its hypotensive effect on hypertensive induced rats (Dimo et al., 1998; Dimo et al., 2001; Dimo et al., 2003) and, its relaxant effect was also showed on isolated rat vascular smooth muscle (Nguelefack et al., 2005). Its antisecretory and antiulcerogenic properties on various gastric ulcers models in rat showed that the methylene chloride extract is the most potent inhibitor of gastric mucosal lesions caused by HCl/ethanol, and that the extract does not possess anti-secretory potential, and tended to increase gastric acidity when gastric outflow is obstructed by ligature; a possible cytoprotective action mediated by the cytoprotective action of endogenous prostaglandins was also demonstrated (Tan et al., 2000). Another team have tested its leaf extract on the snake venoms and found that *Bidens pilosa* leaf extract weakly antagonized the *D. jamesoni* venom and did not potentiate the SAV (Antivenon Serum) (Chippaux et al., 1997).

In this study, we focused our research on one of the empirical uses of it aqueous leaf extract by some tradipracticitionners in Cameroon to facilitate labour during child birth (CSTR/OUA, 1985). According to (Balépa M et al., 1992), 36.4% of pregnant women deliver at home (a cause of mother and child mortality), and 11.7% of the cases occur in the presence of a traditional obstetrician even in urban or in rural zones (INS, 2001). The practice of obstetrics and gynaecology in traditional medicine practice is often used by traditional birth attendants (TBA) or traditional midwives. TBAs activities have been highlighted at WHO seminars because, they play a major role even in big cities (specially in Africa), where maternity hospitals are overcrowded and women in labour have to seek help elsewhere (Sofowora, 1984; INS, 2001).

The activity of *B. pilosa* on the uterine muscle of pregnant women may suggest the presence of active compounds with estrogen-like and/or oxytocin-like activities. Estrogens are steroid hormones with important functions in the regulation of specific sexual processes in females; they affect the myometrium at the end of the pregnancy by decreasing cellular membrane potential which increases the excitability of the uterus (Kurowicka et al., 2005; Riley et al., 2005) and then intervene in the initiation of labour during child birth. In addition, uterotrophic substances such as oxytocin, a neurohypophysial hormone, are frequently used to enhance myometrial contractile activity during labour (Lopez Bernal, 2003), and it is known that the physiological regulation of the oxytocin system is strongly steroid-dependent (Gimpl and Fahrenholz, 2001).

Given the world-wide interest in the regulatory assessment for medicinal products, there is a need to characterize natural substances for rural population. According to the Endocrine Disruptor Screening and Testing Advisory Committee's (EDSTAC), they recommended T1 screening assays for the development of new drugs such as Non Steroidal Anti-inflammatory Drugs (NSAIDs) (Features, 1998). The *in vivo* three days uterotrophic bioassay using prepubertal mice has been used to determine the estrogenic activity of the aqueous and ethanol leaf extracts of *B. pilosa* on uterine muscle, based on the uterine tissue weight increase after several doses of the test compound (Kanno et al., 2001; Owens and Koëter., 2003). For this set of experiment, the subcutaneous administration, which is one of the two routes of administration validated by the OECD (Organisation for Economic, Co-operation and Development) was preferred to the oral route used by TBAs, because, it has been reported that during the validating protocols conducted by several laboratories, no protocol or route of administration was clearly superior in the dose-response experiments, and that the subcutaneous results on prepubescent were near those on ovariectomized rats (OECD, 2003; Owens and Koëter., 2003; Kanno et al., 2003b). In addition, isometric contraction of isolated primed oestrogenized uterine strips using the organ bath experiment was used to evaluate the plant drug/hormone-like effect and to set priorities for additional testing (Arteche et al., 1997; Greim, 2004).

In the present study, we investigated the estrogenic/uterotrophic (*in vivo*) and oxytocic/uterotonic (*in vitro*) effects of the aqueous and ethanol leaf extracts; we then proceeded to perform further experiments on the effect of the aqueous extract of *B. pilosa* leaves (which showed a more potent uterotonic activity) on the contractile uterine in rats, in the presence of some modulators.

Material and Methods

Collection and Preparation of Aqueous and Ethanol Extracts

B.s pilosa plants were collected from Yaounde's suburb (Nkolbisson) from July to November, 2004. A voucher specimen (N° 65112/HNC.) was deposited in the National Herbarium of Cameroon and authenticated by Professor Sonke Bonaventure, University of Yaounde I. Plants were dried and extracted in accordance with international herbal rules in traditional medicine (Drugs, 1995). Following information obtained from traditional birth attendants (TBA), two hundred grams of sun dried leaves of *B.s pilosa* were boiled in one litre of water for twenty minutes. The liquid obtained was freeze-dried giving 38g of a brownish powder. A stock solution of 100mg/ml in distilled water was prepared daily because of the high instability of *B. pilosa*'s components

(Brandao et al., 1997). The ethanol extract was prepared by macerating 200g of sun dried leaves for three days in one litre of ethanol (95% purity). After filtering, the collected extract was concentrated using a rotary evaporator giving about 17.25g of greenish dough. A stock solution concentrated at 100mg/ml was prepared daily by dilution with Tween 80 (0.1%).

Animals

Immature female mice (*Mus musculus*) aged between 19-21 days, weighing 12-20g at the beginning of the experiment were obtained from the Centre Pasteur of Yaoundé-Cameroon. Access to food and water by bottle-feed was unrestricted throughout the four days assay. The animals were housed in ambient room temperature and humidity. The assays started two days after their arrival for acclimatisation and they were sacrificed by euthanasia with ethylene ether. Adult virgin female Wistar rats weighting 200-250g were purchased from Janvier Breeding Centre (France). Twenty-four (24) hours before the experiment, they received an intraperitoneal injection of 17^β Oestradiol Benzoate [20μg × Kg⁻¹]. The rats were humanely killed by inhalation of carbon dioxide (CO₂). These animals (mice and rats) were treated following the international ethical conduct of animal's treatment (Guidelines, 2003).

Pharmacological Experiments

Acute Toxicity Test: (LD₅₀)

The acute toxicity test followed the method used by (Chearskul et al., 2004). Seventy (70) mice aged between 5-6 weeks with average weight between 28-35g were divided into twelve groups of five mice receiving an intraperitoneal injection of a single concentration (per body weight) of aqueous or alcohol extract of *B. pilosa*. Two control groups (for each extract) receiving the corresponding amount of vehicle distilled water or Tween 80 (0.1%) were studied. Five single concentrations: 0.78; 1.56; 2.34; 3.13; 3.90 mg/g/day were given once and the death cases were recorded for three days (72 hours).

Uterotrophic bioassay

180 mice were used and divided into groups of six animals. For the aqueous and ethanol extracts, the test groups received 0.25, 0.5, 1.0, 2.0, 4.0, 8.0mg/g/day, respectively; while the control groups received Tween 80 (0.1%) and distilled water at 2.5, 5.0, 10.0, 20, 40.0, 80.0μl/g/day respectively. Six concentrations (0.02, 0.04, 0.08, 0.16, 0.32, 0.64μg/g/day) of Premarin, a standard sulfoconjugated oestrogen solution, were given as positive control, while 2.5, 5, 10, 20, 40 and 80.0μl/g/day, respectively of Olive oil, used as vehicle to the sulfoconjugated estrogen (Yamasaki et al., 2001), were added to the corresponding solutions. Subcutaneous injections of all extracts were administered once a day for three consecutive days, at about 9h ±15min daily. The LD₅₀ of both extracts were calculated using the Spearman-Kärber method (W.H.O., 1981). $\text{Log LD}_{50} = X_{100} \pm (d/n) \cdot (\Sigma r - n/2)$ where X_{100} is the logarithm of the concentration producing 100% of mice death; n is the number of mice used for each concentration; r is the number of dead mice for each concentration; $d = 2.20$ for $p = 0.05$ with an $d.d.l. = \Sigma (n-1)$ including all mice groups except X_0 and X_{100} .

Determination of body Weight and Uterine Weight

Body weight of mice was recorded during the first and the fourth days of the experiments (approximately twenty four (24) hours after the last injection), and expressed as a percentage of the difference of body weight between the first and the fourth days. Mice were humanely sacrificed by anaesthesia with ethylene ether on the fourth day of the experiment. The uterine horns were removed free of ovaries and adhering fats. Care was taken not to lose the uterine luminal fluid. Wet weights were then recorded on an electronic digital balance (AND FX-300 Japan), and expressed as percentage of the uterine wet weight.

Isometric Contraction Measurement

Uterine horns were dissected and mounted in the organ bath containing a Krebs Heinsleit (KH) solution with the following composition (in mM): NaCl 118.4; KCl 4.7; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; C₆H₁₂O₆, 11.1; NaHCO₃ 25.0 and CaCl₂·2H₂O, 2.5 and equilibrated as previously described by (Savineau & Mironneau, 1990; Arteché et al., 1997). After the equilibration period, the preparations were challenged by administration of a maximal effective concentration of acetylcholine (Ach), 100μM. The Ach-induced contraction was considered as a reference standard contraction and used to normalize the following contractile responses. Uterine strips were then washed 3 times with fresh KH solution and kept not stimulated until stable and regular spontaneous contractions appeared for a further 30-min period before tests. For each experiment, a

ring obtained from the same horn was used as a paired temporal control. Stock solutions of 25mg/ml per extract were prepared daily. Different concentrations of aqueous and ethanol stock solutions were cumulatively injected in the bath at ten minute intervals for final concentrations of 0.03; 0.09; 0.22; 0.47; 0.97; 1.97; 3.97mg/ml.

For this set of experiments, three parameters [the amplitude of spontaneous and transient contractions (named oscillations), the amplitude of the tonic contraction and the rate of oscillations] were selected, measured and calculated in order to characterize the effect of *B. pilosa* extracts on the myometrial tissue. These parameters were calculated as follows:

$$\begin{aligned} \text{Amplitude of oscillations:} &= [(A_c - A_0) / (A_{Ach} - T)] / 100 \\ \text{Tonus:} &= [(T_c - T_0) / (A_{Ach} - T_0)] / 100 \\ \text{Rate of oscillations:} &= [(F_c - F_0) \times 100] \end{aligned}$$

A_c = amplitude recorded during *B. pilosa* cumulative addition in the bath in presence or in absence of the pharmacological drug at c concentration.; A_0 = amplitude recorded after the equilibrating period which follows wash of the reference Ach contraction (control strip) and after the effect of a given pharmacological substance (treated strip); A_{Ach} = amplitude of the reference contraction of Ach 100 μ M; T = basal tonus recorded before the reference Ach contraction.; T_0 = basal tone recorded after the equilibrating period who follows wash of the reference Ach 100 μ M (control strip) and after the effect of the pharmacological substance (treated strip); F_c = Rate recorded during *B. pilosa* cumulative addition in the bath in presence or in absence of the pharmacological drug at c concentration.; F_0 = Rate recorded after the equilibrating period which follows wash of the reference Ach 100 μ M (control strip) and after the effect of a given pharmacological substance (treated strip).

Chemicals

Premarin, a sulfoconjugated oestradiol, was purchased from Wyeth-Medica (Newbridge-Ireland); 17 β -Oestradiol benzoate was purchased from Sigma Aldrich chemicals (France).

Data Analysis

Data obtained from the *in vivo* and *in vitro* tests between control and treated groups were analyzed by a multiple comparison analysis using a One-Way ANOVA: Duncan or Tukey tests and the difference between two means were assessed by the t -test or Mann-Whitney test where appropriate. For the uterotrophic analysis, the five concentrations of *B. pilosa*'s extracts were compared with the vehicles and Premarin treated groups. Uterotonic analysis were assessed by comparing *B. pilosa* extracts (ethanol and aqueous) with vehicles. Body weight was reported as the mean \pm S.E.M for three replicates. The significance was determined at $P \leq 0.05$. All statistics were performed using Sigma Stat version 2.0 and curves draw using Sigma plot 9.0.

Results

In Vivo Study: Uterotonic Effect

Acute Toxicity: LD₅₀

From the intraperitoneal acute toxicity study of both extracts of the leaves of *B. Pilosa* in mice, the LD₅₀ of the aqueous and ethanol extracts were 12.30g/kg and 6.15g/kg respectively.

Mice Body Weight

Compared with negative control, both *B. pilosa* ethanol and aqueous leaf extracts effects were similar and induced significant ($P \leq 0.05$) variations in mice body weight [Figure 1_{B&D}] and the effect produced by the aqueous extract was lowest in general, even at the maximal effect induced. On contrary to the aqueous extract, the ethanol extract, in spite of the general decreased effect observed, at concentrations up to 500 μ g/g/day, we noted a body weight increase higher than that of the negative control at corresponding concentrations. However, compared with the standard [Premarin], mice treated with both aqueous and ethanol extracts [Figure 1_{C&D}], while presenting same variations, showed significant ($P \leq 0.05$) body weight increase with peak values at 0.5mg/g/day (22.8 \pm 0.76%, $P \leq 0.05$) for the ethanol extract and at 1mg/g/day (14.7 \pm 0.84%, $P \leq 0.001$) for the aqueous extract. Up to 4mg/g, a concentration-dependent body weight loss followed by an increase at 8mg/g was observed. However, these values were inferior to those observed at low concentrations of ethanol (+6.71 \pm 0.61%; $P > 0.05$) and aqueous (-2.26 \pm 0.57%, $P > 0.05$) extracts. Comparison of the effects of the two extracts showed that they are both efficient at low concentrations but the ethanol extract induced the highest body weight gain during the experiment.

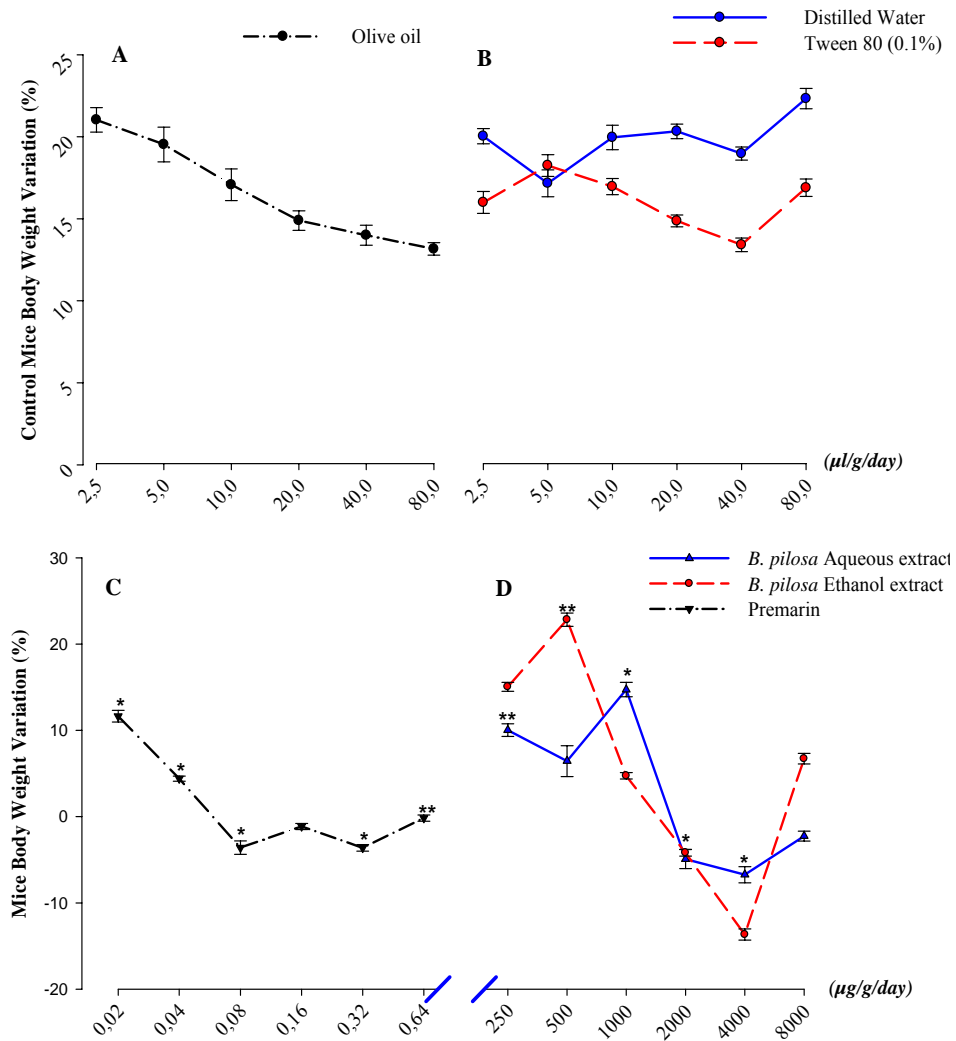


Figure 1: *In vivo* effects of *Bidens pilosa* leaf aqueous and ethanol extracts on immature mice Body Weight. Representative curves of vehicles (negative controls): olive oil (A), distilled water and Tween 80 (B), and representative curves of Premarin (positive control) (C) and *Bidens pilosa* aqueous, ethanol extracts (D) effects on the mice body weight variation during the four day uterotrophic assay. The significance of the extracts effects was expressed versus the positive control (Premarin). * $P \leq 0.05$; ** $P \leq 0.001$.

Uterine Wet Weight

Both ethanol and aqueous extracts of *B. pilosa* administered to immature female mice from 0.25-8mg/g/day tended to increase uterine wet weight ($P \leq 0.05$) with peaks of $0.20 \pm 0.04\%$ ($P \leq 0.001$) and $0.24 \pm 0.001\%$ ($P \leq 0.05$) at 1mg/g/day and 0.2mg/g/day respectively [Figure 2_b]. Higher concentrations ($>2\text{mg/g/day}$) showed a marked decreasing effect of the aqueous extract. Though the ethanol extract exhibited a decreased effect, its values were similar to those observed at 1mg/g peak value. This suggested that increased

uterine weight gain occurred at low and high concentrations of aqueous and ethanol extracts of *B. pilosa* leaf. The three vehicles tested showed no significant ($P>0.05$) variation on mice uterine wet weight [Figure 2_{A&B}]. Comparatively to the positive control [Figure 2_C] which caused a high significant ($P\leq 0.001$) uterine wet weight gain of $+0.62\pm 0.07\%$ at the concentration of $0.64\ \mu\text{g/g}$, and the negative control groups [Figure 2_B], *B. pilosa* extracts showed significant ($P\leq 0.05$) weak uterotrophic activity on mice uterine muscle. These results indicated an uterotrophic effect of *B. pilosa* leaf extracts on the mouse uterine smooth muscle.

The maximal percent increase in both water and ethanol extracts were 38.01 and 31.73% respectively, at concentrations of $2000\ \mu\text{g/g/day}$ and $1000\ \mu\text{g/g/day}$ respectively (concentrations producing the greatest uterotrophic response), equivalent to approximately 1/3 the highest uterine increase of the standard Premarin, at concentration of $0.64\ \mu\text{g/g/day}$.

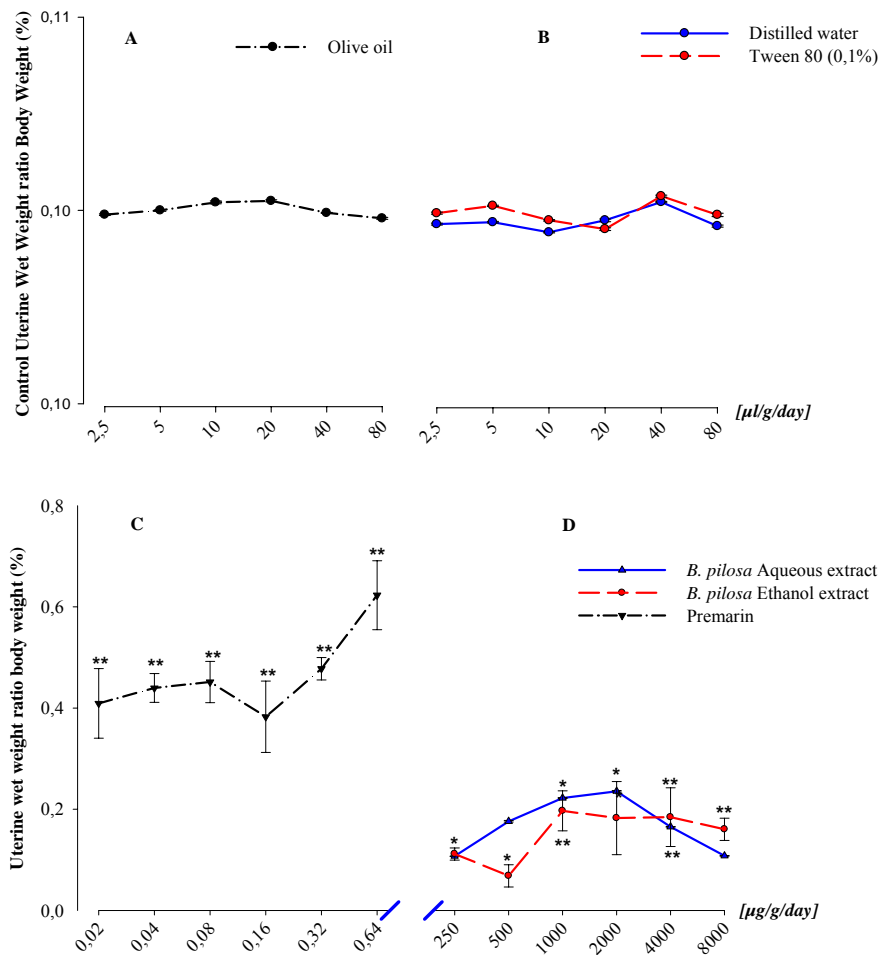


Figure 2: *In vivo* effects of *Bidens pilosa* leaves aqueous and ethanol extracts on immature mice relative uterine wet weight. Representative curves of vehicles (negative controls): olive oil (A), distilled water and Tween 80 (B), and representative curves of Premarin (positive control) (C) and *Bidens pilosa* aqueous, ethanol extracts (D) effects on the mice body weight variation during the four day uterotrophic assay. The significance of the extracts effects was expressed versus the positive control (Premarin). * $P\leq 0.05$; ** $P\leq 0.001$.

In Vitro Study: Uterotonic Effect Mechanical Contractile Induced Effect of *Bidens pilosa* Aqueous and Ethanol Extracts

To evaluate the difference in uterotonic potency between both extracts, the same concentrations of the aqueous and ethanol extracts of *B. pilosa* were tested on the uterine strips. We observed that both leaf extracts generally induced concentration-dependent increases on selected parameters of the uterine strip. For high *B. pilosa* concentrations, the muscle exhibited a rapid contractile response characterized by a brief steady state, followed by a decrease with spike-types which could become a stable steady state with some rhythmic oscillations (aqueous extract) or could reach the level of the basal tonus (ethanol extract) [Figure3a].

The aqueous extract showed highest increases on the three parameters studied. Its maximal effect on the amplitude (+37.61±0.15%) and the rate (+186.22±0.89%) of contractions were observed at the concentration of 1.97 mg/ml. While the maximal tonus increased (+86.21±0.89%) was observed at the concentration of 3.97mg/ml [Figure3b_B]. The effects of the ethanol extract on the amplitude [Figure3b_A] and the rate [Figure3b_C] of oscillations were more complex and clearly biphasic according to the concentration used. Taken together and in comparison of the EC₅₀ of each extract on the different parameters, we noted in general, a significant highest activity (P≤0.05) of the aqueous extract on the amplitude of contractions while, the ethanol extract showed a significant (P≤0.05) superior effect on the tonus of the uterine spontaneous contractile activity. The aqueous extract also showed a higher but non significant (P>0.05) effect on the rate of oscillations contrary to the ethanol extract which presented a lower but significant EC₅₀ value [Table 1].

Table 1: EC₅₀ of *in vitro* effects of aqueous and ethanol extracts of *Bidens pilosa* leaves on the amplitude, the tonus and the rate of rat primed oestrogenized uterine strip contractile activity. *P≤.05; ***P≤.0001.

<i>Bidens pilosa</i> extract :	EC ₅₀ (mg/ml)		
	Amplitude of oscillations	Tonus	Rate of oscillations
Aqueous	0.44 ±0.10*	4.08 ±0.30***	1.21 ±0.25
Ethanol	1.7 ±0.24	0.34 ±0.09*	1.98 ±0.69*

Discussion

Based on the conclusions of previous toxicological results on mice and rats which determined the LD₅₀ <5mg/kg as highly toxic and LD₅₀ >5g/kg as weakly toxic, (Diezi, 1989), and the values of the aqueous and ethanol *B. pilosa* leaf extracts obtained, we can thus characterize both extracts as weakly toxic. But when compared with each other, the ethanol extract present a higher lethal effect than the aqueous extract. This result implies that, as regards of the ethanol extract, a normal adult weighing about 60kg will need to absorb 60×6.15g = 369g to obtain a lethal effect. Yet this quantity is largely higher than any prescribable maximum effective dose of any drug which is usually 1-2g (adult dose) two or three times daily (Kamanyi, 1992). We can thus conclude the concentrations under investigations may not be toxic.

Our results on the three-day uterotrophic bioassay showed a body weight gain in mice at low concentrations but a weight loss at high concentrations. We also observed a general uterine wet weight increase for both ethanol and aqueous extracts. Different laboratories during assessments on uterotrophic protocols (Kanno et al., 2001) have demonstrated that, the three-day bioassay on immature rodent showed no consistent correlation between the body weight gain and the uterine weight gain; and that the body weight adjustment had relatively little impact on the evaluation of uterine weight in these studies. This assertion was verified in our results related to the maximal effect observed on the animal body weight gain and on the uterine wet weight increase occurred at different but low concentrations for both aqueous and ethanol extracts. In reference to previous reports, the uterine weight increase is a fundamental and early marker of the female to sufficient exposure to estrogen agonists. The response begins with the essential interaction of the estrogen with a high-affinity receptor in uterine tissues that initiates a series of responses culminating in the uterine weight increase, which express a combination of water imbibition in the tissue and the uterine lumina, to

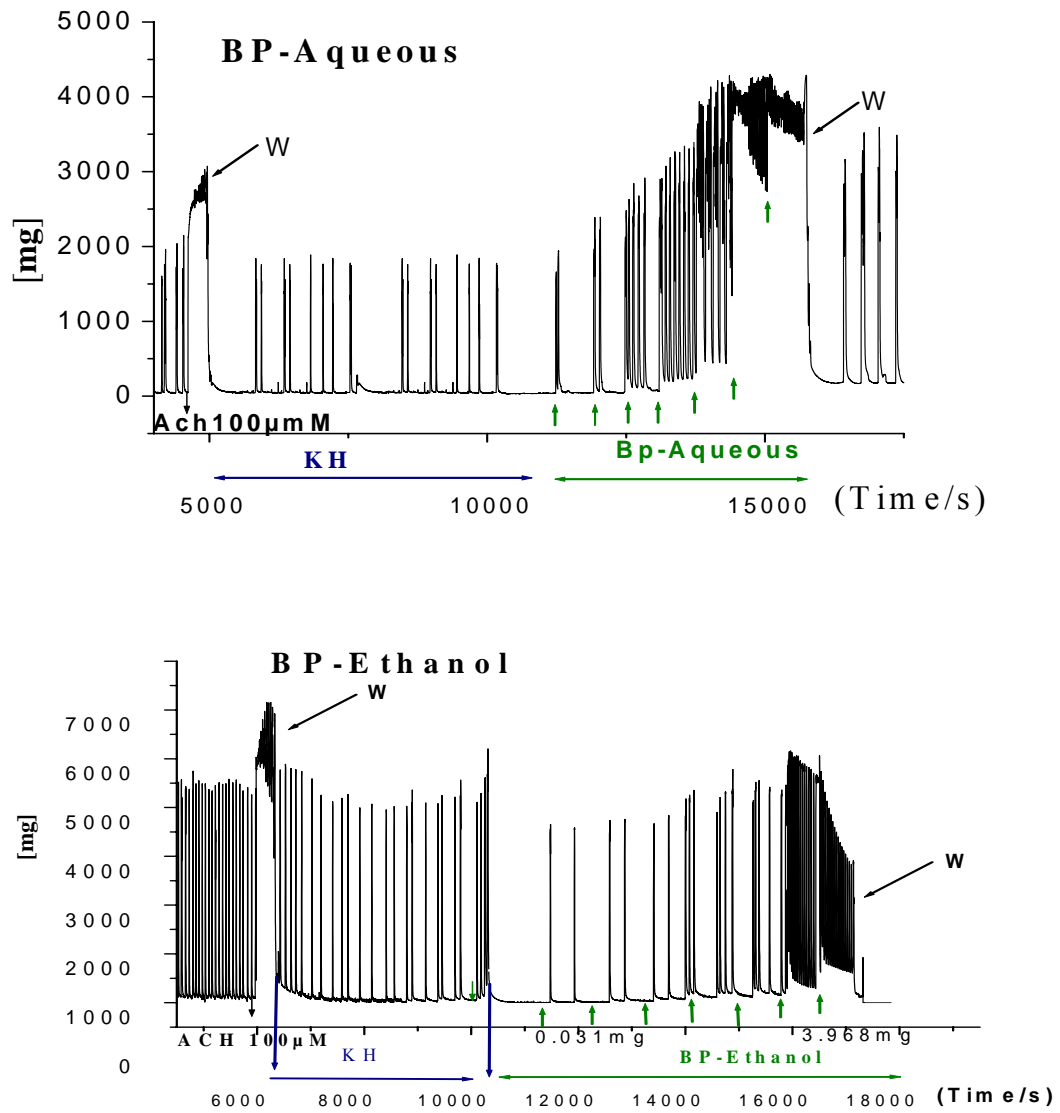


Figure 3a: Original recordings of *in vitro* effects of aqueous and ethanol *Bidens pilosa* leaves extracts on the contractile activity of rat primed estrogenized uterine strip. Seven different volumes of *Bidens pilosa* aqueous and ethanol extract were injected in the organ bath in presence of Normal Krebs solution cumulatively at 10 minutes intervals, respectively. Contractions are expressed in mg (ordinate). Each concentration is represented by an arrow. Ach = acetylcholine, W= wash with Normal Krebs [2.25mM].

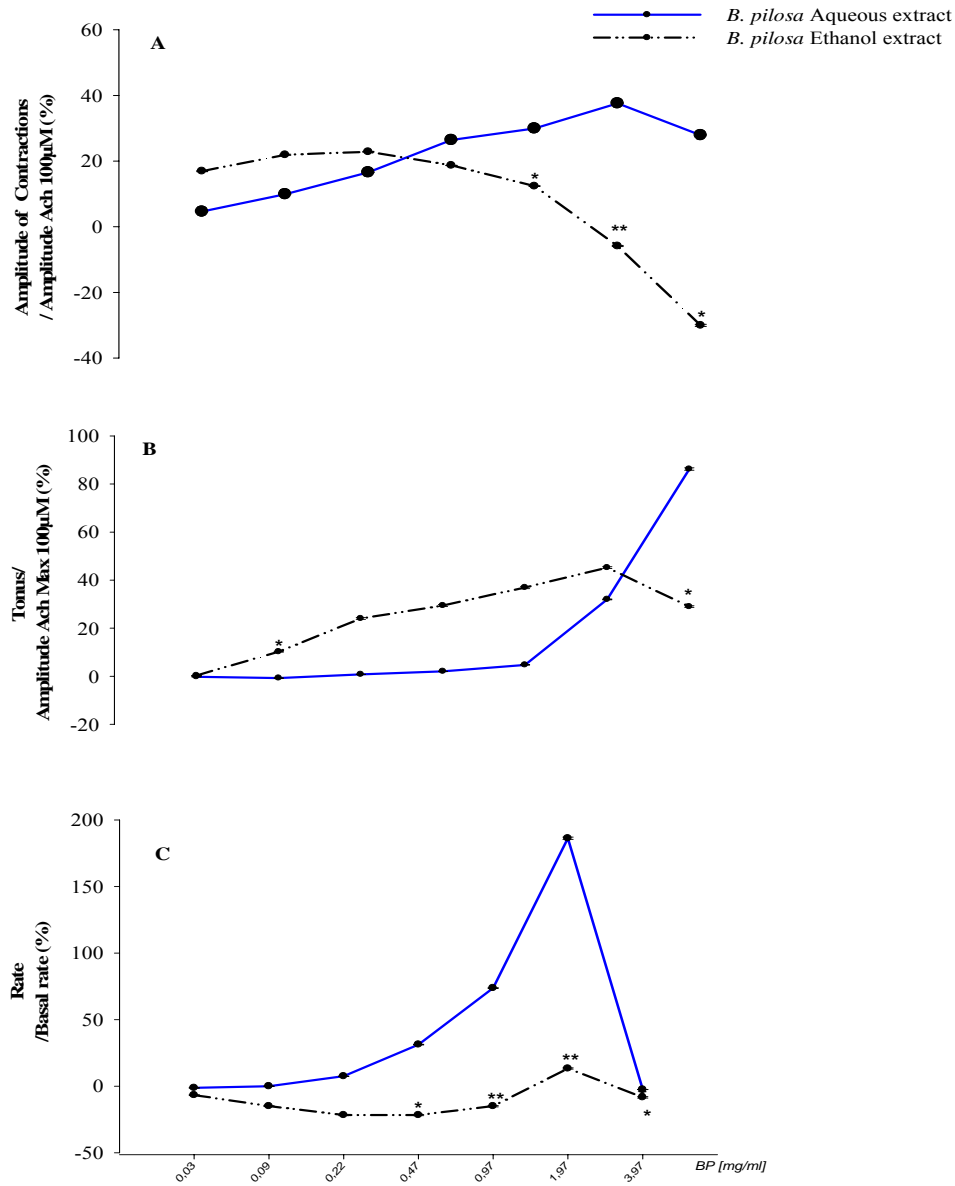


Figure 3b: Curves of *in vitro* effects of aqueous and ethanol *Bidens pilosa* leaf extracts on the amplitude (A) the tonus (B); and the rate (C) of rat primed estrogenized uterine strip contractile activity. ($n=6$) * $P \leq .05$; ** $P \leq .001$. The P value was determined versus the effect of aqueous extract in Krebs solution (standard).

the cellular hyperplasia (Kanno et al., 2001; Kanno et al., 2003b), and to microvascular permeability which may be mediated by growth factors (Chearskul S. et al., 2004). The uterine wet weight increase observed in presence of *B. pilosa* aqueous and ethanol extracts, is due to similar mechanisms, and expresses an uterotrophic effect, which may thus result from the effect of some oestrogenic compounds present in the plant extracts at the level of the uterine muscle. Previous studies on the effect of the aqueous and alcoholic extracts of some tropical plants such as: *Trifolium pratense* (red clover) on the ovariectomized rat (Burdette et al., 2002) traditionally used as *B. pilosa*, to enhance labour during childbirth; *Angelica sinensis* (Circosta et al., 2006), *Morinda citrifolia* (Chearskul S. et al., 2004), mixture of *Aloe buettneri*, *Dicliptera verticillata*, *Hibiscus macranthus* and *Justicia insularis* (P.B. Telefo et al., 2002) used to alleviate menstrual disorders and to cure infertility, have demonstrated that the uterotrophic activity observed could be caused by oestrogenic-like compounds presents in plant extracts. On the other hand, *B. pilosa* at low concentrations displays estrogenic activity that is not observed at higher concentrations which seems to depress the uterotrophic response to below the control levels. Conclusions of previous studies have mentioned that the uterotrophic and the estrogen agonistic effect presented by various natural estrogens [genistein (GN), daidzein (DN), and coumestrol (CN), etc], occur at low doses but not at higher doses (Hideki Wanibucchi et al., 2003; Owens et al., 2003; Yamasaki et al., 2003). Our results also confirm the calabrese's concept of hormone-like biphasic dose response model characterized by a low-dose stimulation which usually present a modest response (30-60% vs. control) and a high-dose inhibition (Calabrese EJ. and Baldwin, 2003; Chearskul S. et al., 2004); this gives proof that *B. pilosa* leaf active compounds act after a uterine receptor binding.

The *in vitro* test performed on primed-oestrogenized isolated uterine strips confirms the hormone-like (estrogenic-like) effects of both aqueous and ethanol extracts, and clearly showed their different potencies and characteristics on the uterine muscle. Each extract presents a different action on the parameters chosen to characterize *B. pilosa* activity. Their significant effects on the amplitude and the tonus are quite opposite as concentrations increased, with a highest effect at low concentrations for the ethanol extract and a highest effect of the aqueous extract at high concentrations. This could be related to the amount of *B. pilosa* active chemicals which have positive effects on those parameters. On the other hand, the lack of the ethanol effect on the rate could imply a lack of biological activity of the extract on the enhancement of the rate of contractions. These differences could be due to the existence of active substance(s) in *B. pilosa*'s leaves, which have a variable solubility coefficient (difference in polarity) of the molecules extracted with the solvent used for extraction (water and ethanol). Natural estrogenic-like compounds are generally more extractable in ethanol than in water (Circosta et al., 2006). Earlier phytochemical screenings of different extracts of *Bidens pilosa*'s leaves revealed the presence of biological components such as polyacetylenes and flavonoids in abundance (Alvarez et al., 1996; Chang et al., 2004). Some natural flavonoids have been tested on human and rat myometrium and presented a weak estrogenic activity (Gilbert et al., 1999; Kim et al., 2002; Kim et al., 2005), and a relaxant effect (Revuelta et al., 1999). This dual effect is in accordance with our results could be due to the nature of solvent used for extraction or the instability of polyacetylene bioactivity (Krettli et al., 2001). Earlier researchers have reported that, estrogens possess some oxytocic effect and hence may be involved in increasing uterine contractility (G. Tafesse et al., 2005). The oxytocin hormone was reported to biologically act via binding to a uterine membrane receptor, (OTR), with seven (7) transmembrane domains, and belongs to the class I G protein-coupled receptor (GPCR) family, and thereby stimulates a cascade of events leading to the contraction of the uterine contractile proteins (actin and myosin) (Keena McKillen et al., 1999; Gimpl and Fahrenholz, 2001; Muller et al., 2006). Similarly, the increase of the uterine induced contractile activity observed in our results could be due to the presence of oxytocic-like compounds acting via the same pathways. The comparison of the oxytocic-like activity of both aqueous and ethanol extracts, on all parameters, showed a highest activity of the aqueous extract on the primed estrogenized uterine muscle. When considering the phytochemical composition of the different plants extracts of *B. pilosa* extracts, all are composed of common chemicals such as phenylheptatryine, quercetin, ferulic acid, caffeic acid, flavone glycosides, and minerals. These compounds have been reported to acts severally and/or jointly on the biological activity of plant extracts (Brandao et al., 1997; Lic. Humberto et al., 2001). During the evaluation of the antioxidant activity of some natural compounds by (Usami et al., 2004), they found that the water-soluble phase showed stronger activity than the lipo-soluble phase, which was mainly due to the stronger effect of ferulic acid, caffeic acid and quercetin. Therefore, the biological active substances of *B. pilosa* leaf extracts could be essentially due to those compounds which present a higher uterotrophic activity in the aqueous extract.

In conclusion, *B. pilosa*'s leaf extracts induced a biological agonist reaction on the uterine muscle, related to the presence of active compounds, which possess estrogenic-like and oxytocin-like activity. The aqueous extract showed a higher uterotrophic/estrogenic effect while the aqueous extract showed a higher uterotonic/oxytocic-like effect. Those characteristics are probably responsible for its empirical use as a labour enhancing substance. The present work represents the first investigation of the uterotrophic and uterotonic effects of *Bidens pilosa* on the myometrial smooth muscle. However, further electrophysiological experiments

are going on to clearly define the proposed signalling pathway(s) and phytochemical analysis is required to isolate active components responsible for the observed uterine muscle contractile effect.

Acknowledgments

The authors thank Mrs Huguette Crevel from the Laboratoire de Physiologie Cellulaire Respiratoire; INSERM U885 (Bordeaux, France) for her technical assistance, Dr Pascal Wafo from the department of chemistry (E.N.S. Yaounde) for plant extractions, and Mr Eboñe Thomas for plant collection. F. Longo was a recipient of the French Cooperation Organism (EGIDE).

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