
The oxytocic properties of *Luffa cylindrica* (L.) M. Roem. and *Bidens pilosa* L., traditionally used medicinal plants from western Uganda

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Abstract

The usage of medicinal plants in childbirth in Uganda is a long standing tradition. Over 80% of Ugandan women have childbirth at home and herbal remedies are administered to complete the process of childbirth. The herbs are administered to hasten the labour process (oxytocics), expulsion of retained placenta and control postpartum bleeding. This indigenous knowledge in herbal medicines used during childbirth is not well documented and scientifically validated. The ethnobotanical study was conducted between 2000 and 2003 in Bushenyi and Kasese districts in western Uganda. The aim of this study was to validate the claimed uses of *Bidens pilosa* L. and *Luffa cylindrica* (L.) M.J. Roem. in inducing labour during childbirths in western Uganda. The *in vitro* experiments using the rat uterus showed that the aqueous leafy extracts of *B. pilosa* and *L. cylindrica* increased rat uterine motility suggesting that they are oxytocics. The aqueous leafy extract of *B. pilosa* *in vitro* experiment using the rabbit jejunum further indicated that the extract can cause contraction. The bioactivity of *B. pilosa* and *L. cylindrica* is a pointer to the therapeutic uses of herbal remedies in childbirth.

Key words: childbirths, medicinal plants, uterine motility

Introduction

In Uganda, complications resulting from reproductive health-related conditions such as maternal mortality and

morbidity are a major problem among local communities (NHP, 1999; HSSP, 2000). The national maternity mortality average is 506/100,000 (HSSP, 2000) and has remained stable for the last 10 years while the infant mortality is 89.4/1000 (CIA, 2002). The Ugandan population having access to basic health services within 5-km walking distance is 49% and most health centres are confined to urban areas. More than 60% of mothers are not attended to by trained health personnel during childbirth in Uganda. The medical doctor to patient ratio is 1 : 20,000, compared to the ratio of traditional healer to population of between 1 : 200 and 1 : 400 (WHO, 2002b). The Uganda's population of over 24 million, high population growth rates of about 3.0% and the fertility rate close to 7.0 makes it impossible for the proper provision of basic healthcare facilities (CIA, 2002; UNAIDS, 2002; Kelley, 2003). Although the majority of the women receive antenatal care (91%), only 37% are supervised by the trained health workers (UDHS, 2000). Kamatenesi-Mugisha (2005) reported that in rural western Uganda, close to 80% of pregnant women deliver at home. To complete the processes of child bearing herbs are mainly given by the traditional birth attendants (TBAs) and other knowledgeable elderly women and mothers in law in the rural communities (Neema, 1999; Kamatenesi-Mugisha, 2003, 2004, 2005). Thus, this study screened the aqueous crude herbal drugs from *Bidens pilosa* and *Luffa cylindrica* by carrying out bioactivity tests on the rat uterus motility and rabbit jejunum motility.

Oxytocics which are agents that promote uterine contractions, are used for inducing childbirths in western medicine (Katzung, 1992; Roper, 2001; Hardman, Limbird & Goodman-Gilman, 2001). On the other hand, in

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traditional medicine, medicinal plants are used to stimulate and induce childbirths. Thus in this study, some of these plants were screened and the solvents used in extraction were ethanol and water. Water was used as a solvent in extraction because the local people administer most of their medicine in aqueous form. However, ethanol may be used as a solvent because at times traditional healers use locally made beer or fermented porridge or milk to extract and administer herbal drugs. More to that ethanol is safe even when consumed orally by humans at moderate concentrations. Furthermore, in terms of polarity, ethanol between 80% and 95% is the best safe substitute because it extracts most of the compounds that would be extracted with water and some other organic solvents. As this research was conducted to validate the claims of indigenous knowledge, the initial screening was by ethanolic plant extracts but for final ethnopharmacological screening experiments, aqueous extracts were used.

The specific objective was to examine the oxytocic activities of *L. cylindrica* and *B. pilosa* medicinal plants in relation to their claimed traditional uses in reproductive health care.

Materials and methods

The research methods were mainly informal conversations and semi-structured interviews of group and individual focused group discussions and field visits were conducted to generate the ethnobotanical data (Martin, 1995). To reach the traditional medical practitioners (TMPs) and TBAs, the local authorities (Local Council Leaders) and herbalists associations in villages were consulted. This ethnobotanical information was collected through visiting traditional healers to document indigenous knowledge regarding medicinal plants used, ailments treated, gender and socio-cultural aspects. The medicinal plants voucher specimens were collected, documented and identified in the Makerere University Herbarium.

Collection, preparation and extraction of the plant material

The *L. cylindrica* (L.) M.J. Roem. plant materials were collected in Kirugu Parish in Kichwamba subcounty in December 2000 and *B. pilosa* L. leaves were collected between March and April 2001 from Kyeibanga parish in Kitagata subcounty in Bushenyi District, western Uganda. The ethnopharmacological screening was conducted between 2001 and 2003 in the University of Nairobi, Kenya.

The plant parts used in folk medicine were collected and air-dried at room temperature for at least 2–3 weeks. The dried plant materials were pounded in a metallic mortar to a fine powder. The pounded plant materials for the preliminary screening were extracted with 80–95% ethanol and prescreened. For final testing of the claimed tocolytic and oxytocic medicinal plants, water samples were prepared by freeze drying using a freeze drier. To prepare the water extracts, weighed amounts of plant material were put in conical flasks and boiled in water for 10–15 min, cooled and filtered using the pressure pump and others using a funnel and cotton-wool. The filtrate would be stored at -20°C for later freeze-drying.

Preparing the rat uterus

Young wistar virgin female rats weighing between 120 and 200 g were used in the uterine motility experiments. The sensitivity of the uterus was increased by a subcutaneous injection of stilboestrol (0.1 mg kg^{-1}). The rats were left for 48 h after which these young rats were killed by a blow on the head. The uterus was carefully dissected in a Petri dish of de Jalon ringer solution at 32°C . The uterine horns separated from the animal just below the ovaries were cleaned and any extraneous fat and connective tissue was removed. The horns were then separated at the bifurcation, yielding two preparations. One preparation was taken and mounted in the aerated (95% O_2 and 5% CO_2) organ bath at $32\text{--}37^{\circ}\text{C}$, for at least 30 min to normalize before adding the plant crude drugs and standard drugs (oxytocin) so that the spontaneous activity could be deduced. To minimize the experimental errors of dilution, the known weighed drugs were transferred into the test tubes and dissolved in de Jalons solution as they were water extracts. In each consecutive experiment, new de Jalons solution and crude plant drug were freshly made.

The plant drug or standard drug (for control) was then injected into the organ bath with the tissue and the 7050 microdynamometer recording machine fixed on it. The transducer and writing lever were translating the tissue movements on the 7050 microdynamometer onto recording graph paper (Fig. 1). The tissue was always washed with de Jalon ringer solution every time injected drug (or set drugs) recording was made before another drug (or set drugs) was introduced. The time of tissue washing varied based on the behaviour of the drug on the tissue. The washed tissue in the organ bath was left to normalize before addition of another drug. The tempera-

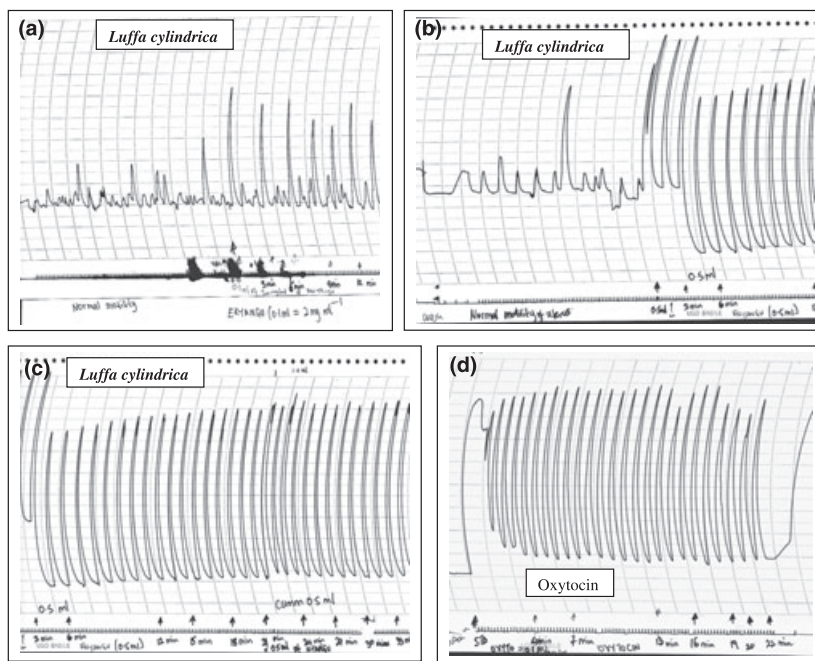


Fig 1 The effect of aqueous extract *Luffa cylindrica* on the rat uterine motility: (a) *L. cylindrica* at 0.29 mg ml^{-1} ; (b) *L. cylindrica* at 1.43 mg ml^{-1} ; (c) *L. cylindrica* at 2.86 mg ml^{-1} ; (d) oxytocin of $1.0 \text{ } \mu\text{g ml}^{-1}$.

ture of the organ bath containing the tissue was always maintained between 32 and 37°C.

Procedure of preparing the rabbit jejunum

A female rabbit purchased from the farm owned by a farmer/animal house attendant in the department of Pharmacology and Therapeutics, was killed, the abdomen opened, the caecum was lifted forwards and the ileum was found joined at the back of it. The intestine was cut at a point 5–10 cm below the stomach and the length taken from here downwards to the caecum and placed in a dish containing Tyrode's solution. The tissue did not require intensive washing because the rabbit intestines are wider than guinea-pig intestines and partly because its spontaneous activity causes it to clear itself even after it is severed. Great care was taken to avoid damaging the gut muscle. It was handled with fingers rather than with forceps. The mesentery was trimmed away and pieces were cut from the length of jejunum, as required starting above Peyer's patch. A portion of 2–3 cm in length, free from mesenteric attachments was cut and tied with thread at each end, taking care to ensure the jejunum was left open and that the threads did not close the lumen. Each piece was then mounted in an organ bath and aerated with a mixture of 95% oxygen and 5%

carbon dioxide. Once mounted, the piece of the rabbit jejunum contracted rhythmically and in a regular manner. After normalizing the tissue for about 30 min, the herbal drugs were introduced as well as standard drugs to act as controls. The pendular movements of the rabbit jejunum were translated by the transducer attached to the 7050 microdynamometer recording machine recording paper.

Procedure of preparing the aqueous herbal drugs

Exactly 100 mg of each tested sample were weighted using a weighing machine of up to four decimal places. The 100 mg of aqueous prepared herbal drugs were dissolved in 5 ml of de Jalons solution for rat uterus or Tyrode's solution for the rabbit jejunum experiments. Serial dilutions were further made from the stock solution where necessary. To draw the specified amount of the herbal drug to introduce to the organ bath, 1 ml new disposable syringes were used. The tissues were mounted in the bathing solution of c. 7 ml for both the uterus and jejunum tissues. Therefore, the actual concentration of the herbal extract was that particular concentration drawn using the syringe plus the organ bath dilution effect. Thus 20 mg ml^{-1} was divided by 7 ml of the organ bathing solution (Table 1).

Table 1 The actual dilutions of the aqueous herbal drugs

Concentration of the stock solution (ml)	Concentration (mg ml ⁻¹)	Final concentration in organ bathe (mg ml ⁻¹)
0.1	2.0	0.286
0.2	4.0	0.571
0.3	6.0	0.857
0.5	10.0	1.429
1.0	20.0	2.857
2.0	40.0	5.714

Results

The aqueous herbal extract of *Luffa cylindrica*

The aqueous herbal drug of *L. cylindrica* was strongly oxytocic and at 0.29 and 1.43 mg ml⁻¹ (Fig. 1a,b) was capable of producing strong uterine contractions. The rat uterine motility caused by *L. cylindrica* at 0.29 mg ml⁻¹ (Fig. 1a) and 1.43 mg ml⁻¹ (Fig. 1b) was notable. Also, the comparison in uterine motility caused by *L. cylindrica* at 2.86 mg ml⁻¹ (Fig. 1c) and oxytocin of 1.0 µg ml⁻¹ (Fig. 1d) showed that *L. cylindrica* was oxytocic. The force and amplitude of contractions was vivid and even stronger than that due to oxytocin. The uterine motility caused by *L. cylindrica* was spontaneous.

The aqueous herbal extract of *Bidens pilosa*

The aqueous extract of *B. pilosa* stimulated uterine motility. At a concentration of 1.43 mg ml⁻¹ of the *B. pilosa* caused spike like movement at intervals (Fig. 2a). At the concentration of 2.86 mg ml⁻¹, the rat uterine motility increased (Fig. 2b) even beyond the recording paper until when the sensitivity was reduced after 14 min. Thus, the aqueous *B. pilosa* increased uterine motility and this im-

plies that it might contain oxytocic constituents. The uterine motility caused by *B. pilosa* was not spontaneous but rhythmic.

Effect of *Bidens pilosa* on the rabbit jejunum

The aqueous herbal extract of *B. pilosa* (2.86 mg ml⁻¹; 5.71 mg ml⁻¹) slightly increased and shifted the baseline upwards of the jejunal motility (Fig. 3a). The standard drug neostigmine increased motility by shifting the baseline. *B. pilosa* alone enhanced the motility and the combination of neostigmine and *B. pilosa* extract further enhanced the jejunal motility (Fig. 3b). This implied that *B. pilosa* increased the jejunum motility. The extract of *B. pilosa* when combined with neostigmine enhanced jejunal motility.

Discussion

In the interpretation of the results from the ethnopharmacological tests, normal motility of the uterus or jejunum was the baseline for that particular tissue in the screening of aqueous herbal extracts and standard drugs as control. The standard drug (control) for motility in rat uterus was oxytocin. The standard drug (control) for motility in rabbit jejunum was neostigmine.

The aqueous herbal extract of *L. cylindrica* is highly oxytocic in that it caused strong contractions at concentrations as low as 0.29 mg ml⁻¹. The force and amplitude of contractions shows that the herbal extract can fasten labour during childbirth. The contractions due to *L. cylindrica* are immediate and stable at longer time intervals of over 30 min as shown from laboratory testing. These laboratory results do conform to the reported disadvantages of using *L. cylindrica* herbal drug by the TBAs.

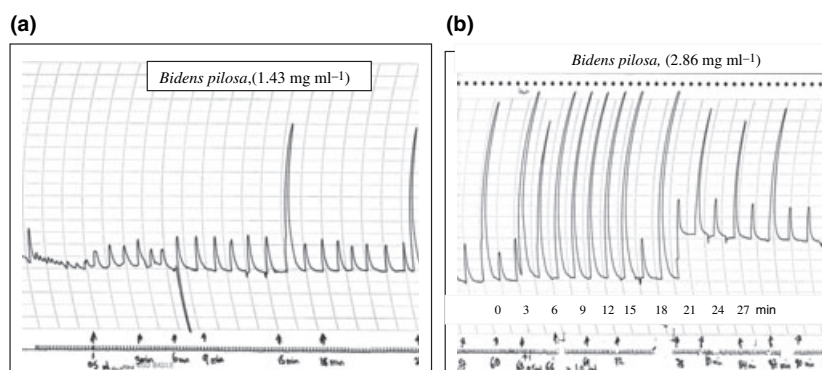


Fig 2 The effect of aqueous *Bidens pilosa* extract on the rat uterine motility: (a) *B. pilosa* of 1.43 mg ml⁻¹; (b) *B. pilosa* of 2.86 mg ml⁻¹.

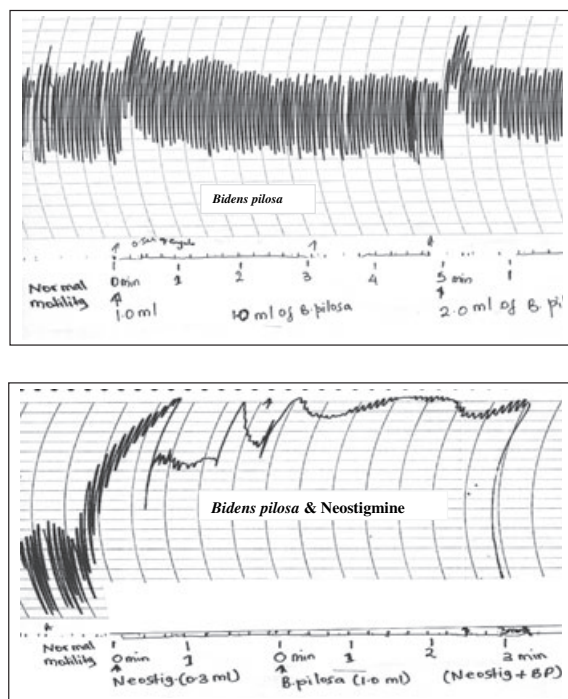


Fig 3 The effect of the aqueous *Bidens pilosa* in the rabbit jejunum motility: (a) *B. pilosa* (2.86 mg ml^{-1} ; 5.71 mg ml^{-1}); (b) jejunum motility comparison of *B. pilosa* (2.86 mg ml^{-1}) and neostigmine ($3 \mu\text{g ml}^{-1}$).

Although the plant can induce labour, there are dangers of rupturing the uterine membranes and other associated problems and complications. The healers reported that the overuse of *L. cylindrica* by women in labour can end up producing children with red eyes or even death. This is a confession from TBAs during ethnobotanical fieldwork. In China, the seeds of *L. cylindrica* were reported to be abortifacient in pregnant mice (Ng, Wong & Yeung, 1992). From the NARPLERT database, the reported research outside Africa about other *Luffa* species like *Luffa echinata* aqueous extract caused smooth muscle stimulation in guinea-pig ileum in both pregnant and nonpregnant rat uterus (Misra *et al.*, 1967; Misra, Mishra & Misra, 1969) and abortifacient use (Saha, Savini & Kasinathan, 1961). The laboratory findings from this study do correspond with the indigenous knowledge and other similar studies carried out on *Luffa* species suggesting that *L. cylindrica* is a potential oxytocic plant.

The aqueous herbal extract of *B. pilosa* increased rat uterine motility. The extract also strongly augmented the

activity of oxytocin. The aqueous extract of *B. pilosa* acts more in augmentation of contractions in jejunal smooth muscle. *Bidens pilosa* has the ability of activating or augmenting exogenous and endogenous oxytocin henceforth, induce labour in child birth and control of postpartum bleeding. This is in line with the TBAs claims of the uses of the plant. In Rwanda, the methanolic extract of *B. pilosa* had weak uterine stimulant effects on the guinea-pig uterus (Chagnon, 1984).

Although herbal remedy usage in hastening childbirth can be lethal to the mother and foetus, the dosage used in pregnancy at the time of initiating, sustaining or augmentation of labour is low. The TBAs have long experience in childbirth and have, overtime, developed means of regulating dosage hence minimizing lethal effects. In childbirth, only a few leaves are squeezed or chewed. In comparison to the induction of forced abortion where large amounts of the plant extracts are orally taken for several days. The plants may cause mild effects when consumed to induce labour and may not pose serious threat of rupturing the uterine membranes depending on the dosage and the experience of the person administering them.

Exogenous oxytocin can initiate or enhance rhythmic contractions at any time. The uterine stimulating agents used most frequently to induce or augment labour (Dudley, 1997) commonly in hospitals as plants are common to TBAs in hastening childbirth. Some plants used in quickening childbirth are used for treating other diseases. *Bidens pilosa* is used as an antimalarial herbal drug. Katzung (1992) reported an eightfold increase in uterine sensitivity to oxytocin during the last half of pregnancy. The use of exogenous oxytocin at any stage may, however, facilitate labour progression. In early pregnancy, as higher doses are required for exogenous oxytocin to have action, the usage of plants may be relatively safe. This may also imply that use of oxytocic medicinal plants for other ailments in pregnancy may be safe depending on the dosage used, the stage of pregnancy and the vitality of the pregnant woman.

On the other hand, the use of exogenous oxytocic plants in rural areas is of concern. In Uganda, maternal mortality rate has remained stable for over 10 years despite the intensive safe motherhood programmes. The fact that 80% of pregnant women in rural areas have childbirth at home and herbs are employed in the childbirth process raises a lot of concern. The use oxytocic herbs to hasten childbirth where monitoring of women in labour is lacking, may cause the rupturing of the uterine membranes or even

cause foetal and maternal death when inappropriate doses are used. This study recommends more investigations in herbal remedies used in childbirths. More research is needed to investigate the receptors that these medicinal plants work on, monitoring of TBAs during antenatal and home deliveries and effects of oxytocic plant remedies on the functioning of other organs including the heart, brain and liver. In addition, the integration of indigenous knowledge into the national healthcare package will foster collaboration, herbal drugs standardization, acceptability and monitoring of TBAs activities, with the purpose of providing quality healthcare service delivery in Uganda and beyond. Additionally, similar scientific investigations of plants used in reproductive health care will improve Uganda's healthcare delivery base to promote safe motherhood, standardization of herbal products and drug development that conforms with World Health Organisation Traditional Medicine Strategy (WHO, 2002a,b) and the African traditional medicine agenda.

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