Ethno-pharmacological screening of *Vernonia amygdalina* and *Cleome gynandra* traditionally used in Childbirth in Western Uganda

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Abstract

Over 80% of pregnant women in Western Uganda deliver at home with the assistance of mainly traditional birth attendants who use herbal remedies to complete the processes of child bearing in the rural communities. In Uganda, complications resulting from reproductive health related conditions such as maternal mortality and morbidity (20.4%) account for number one problem among the disease burden followed by malaria (15.4%). The national maternal mortality average is 506/100,000 and that has remained stable for over the last ten years. Despite the wide usage of herbal remedies in childbirth, this indigenous knowledge is not well documented and the claims not properly validated through scientific scrutiny under conditions mimicking the indigenous methods of use. This paper will discuss the crude aqueous herbal extracts of *Vernonia amygdalina* Del. and *Cleome gynandra* L. that were screened for their bioactivities on the motility of the rat uterus and rabbit jejunum. The ethnopharmacological screening results showed that the aqueous herbal extracts of *V. amygdalina* and *C. gynandra* increased rat uterine motility. In addition, aqueous extracts from *V. amygdalina* caused rabbit jejunum contraction. The aqueous plant extracts of *V. amygdalina* and *C. gynandra* that increased the rat uterine contraction may be oxytocic. Since the usage of herbal medicines offers a holistic approach that is lacking in western medicine, integration and safety aspects of herbal medicine development is a concern to the developing countries and globally.

Key words: medicinal plants, oxytocics, childbirth, western Uganda

1. Introduction

In Uganda, complications resulting from reproductive health related conditions such as maternal mortality and morbidity account for number one problem among the disease burden (NHP, 1999, HSSP, 2000). The perinatal and maternal related conditions (20.4%) being the first, followed by malaria (15.4%), acute lower respiratory infections (10.5%), AIDS (9.1%) and diarrhoea (8.4%) and these together account for over 60% of the total burden. The national maternal mortality average is 506/100,000 (HSSP, 2000) and that has remained stable for the last ten years and infant mortality is 89.4/1,000 (CIA, 2002), excluding home deaths that are not recorded. The Ugandan population having access to basic health services within 5 kilometre walking distance is 49% because most health centres are confined in urban areas. More than 60% of mothers in Uganda are not attended to by trained health personnel during child-birth. In Uganda, there are at least 290 people for one traditional medical practitioner, compared to one western trained medical practitioner for every 10,000 people in urban areas and 50,000 people.
in rural areas (IK Notes, 2003). This is an indicator that the health sector in Uganda is grossly underdeveloped. The Uganda’s population of over 24 million, high population growth rates of about 3.0% and the fertility rate close to 7.0 is a threat to women health and make it difficult for the proper provision of basic health facilities (UNAIDS, 2002, CIA, 2002). On average, the number of children born to a Ugandan woman during her life time is 6.9 which is substantially greater than Kenya’s total fertility of 3.4 and Tanzania’s rate of 5.3 (CIA, 2002; Kelley, 2003).

The research findings in Chapter two reveal that over 80% of the mothers use traditional medicine to provide health care for themselves and children. The treatments of most of the ailments that women suffer especially in rural areas depend on herbs first and in case the condition deteriorates, then they seek modern health facilities. Over 80% of pregnant women in Western Uganda have childbirth at home with the assistance from traditional birth attendants (TBAs) who use herbal remedies (Kamatenesi-Mugisha, 2004). For instance, to complete the processes of child bearing, herbs are mainly given by the traditional birth attendants and other knowledgeable elderly women and mothers in the rural communities (Neema, 1999; Kamatenesi-Mugisha, 2002). The use of herbal medicines offers a holistic approach that is lacking in western medicine (Anokbonggo, 1992) and its development is a concern to the developing countries and on global panorama (WHO, 2002a; WHO, 2002b).

Although few studies have been carried out on the pharmacological properties of medicinal plant species such as *Phytolacca dodecandra* and *Solanum terminale* (Anokbonggo, 1974) with respect to reproductive health care in Uganda, the majority of plant species though widely used, have not been well researched. This knowledge in herbal medicines used in inducing labour and inhibiting threatened abortion is not well documented and claims properly validated through scientific scrutiny mimicking the local knowledge. Thus, in this study the crude aqueous extracts of the herbal drugs were screened by carrying out bioactivity tests on the rat uterus motility and rabbit jejunum motility. The herbs used in traditional medicine to induce uterine contractions (the oxytocics also used as abortifacients and in post-partum haemorrhage) and the medicinal herbal drugs that inhibited pre-term labour or threatened abortions and miscarriages (the tocolytics) were studied *in vitro*.

**The Therapeutic Values of Oxytocics**

The oxytocics are agents promoting uterine contractions. Oxytocin is the drug of choice for the induction of labour. Oxytocin is a posterior pituitary hormone that is synthesized in the hypothalamus, transported to the posterior pituitary gland for storage and then released into the circulation (Katzung, 1992). Oxytocin is a peptide secreted by the posterior pituitary gland. Oxytocin elicits milk ejection in lactating women, induces uterine contractions, maintains and augments labour. Oxytocin can be used for the control of postpartum haemorrhage. Oxytocin stimulates both the frequency and force of uterine contractions. The spontaneous responsiveness of the uterus to Oxytocin roughly parallels the increase in spontaneous activity. These Oxytocin effects are highly dependent on oestrogen and the maturity of the uterus. Dysfunctional labour augmentation by Oxytocin is seen more frequently in nulliporous women, where Oxytocin can be used to their advantage to facilitate labour. Oxytocin is useful where there is very prolonged latent phase of cervical dilation, compromised foetal oxygenation due to loss of placenta exchange, incompletely dilated cervix and ruptured membranes. However, indications of induction of labour include situations in which the risk of continued pregnancy to the mother or foetus is considered greater than the risk of delivery or of pharmacological induction. Such circumstances include premature rupture of the membranes, isoimmunisation, intrauterine growth retardation and placental insufficiency (i.e. as in diabetes, preeclampsia or eclampsia pregnant mothers).
However, Oxytocin has also similar potential complications like causing ruptured membranes in case of overuse.

Oxytocin is used for induction and reinforcement of labour in women with mild pre-eclampsia near term, uterine inertia and incomplete abortion and it cannot be used for elective induction of labour in the absence of these indications (Katzung, 1992). During the first two trimesters of pregnancy, the motor activity is very low, but spontaneously the motor activity progressively increases until the sharp rise that constitutes the initiation of labour and delivery (Hardman, et al., 2001).

After delivery of foetus or following therapeutic abortion, it is desirable to have the uterus firm and contracted and this greatly reduces the incidence and extent of haemorrhage. Oxytocin is usually given after delivery to help to maintain uterine contractions and tone. In non-hypertensive patients, ergot alkaloids such as ergonovine maleate (ergotrate) or methylergonovine maleate (Methergine) are used.

Oxytocin also plays an important physiological role in milk ejection. Stimulation of breasts through suckling and mechanical manipulation induces Oxytocin secretion, causing contraction of the myoepithelium that surrounds areolar channels in the mammary gland. The action forces milk from the alveolar channels into large collecting sinuses where it is available for the suckling infant.

Specific Objective
The specific objective of this study was to examine the biological activities of *Vernonia amygdalina* and *Cleome gynandra* medicinal plants commonly used in traditional medicine in reproductive health care.

2. METHODS

Ethnobotanical Data Collection Methods
The research methods used to collect the ethnobotanical information were mainly those that promoted free sharing of information between the researchers, the herbalists and other people in the community. Therefore, informal conversations and semi-structured interviews 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 traditional birth attendants (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 the indigenous knowledge (IK) 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. The main respondents during the study were traditional healers, traditional birth attendants (midwives), women and elderly people.

Collection, Preparation and Extraction of the Plant Material
The medicinal plants studied in this paper *Cleome gynandra* L. (Capparaceae) and *Vernonia amygdalina* Del. (Asteraceae), have been used traditionally to induce childbirth hence hasten labour process. The plant voucher specimens for identification were collected from Kirugu Parish in Kichwamba sub-county, Bushenyi District in western Uganda between August and December 2000 and were identified in Makerere University Herbarium. The plant materials for scientific validation of *Cleome gynandra* (roots) and *Vernonia amygdalina* (leaves) were
collected from Kichwamba and Kitagata sub-counties in Bushenyi district between March and April 2001 respectively.

The different plant parts used in folk medicine were collected and air-dried at room temperature for at least one month. The plant parts collected were according to the traditional healers’ prescriptions. The dried plant materials were pounded in a metallic mortar into fine powder. Some other plant materials were grinded using an electric grinder. The pounded plant materials were extracted with 80-95% ethanol and water extracts were prepared and then freeze-dried using a freeze drier in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Nairobi. However, due to mechanical breakdowns, low freeze-drier mortar capacity and great demand of the freeze-drier; it was not possible to freeze dry all the samples. Thus, more water extracts were prepared and then oven dried at 40 0C to 45 0C in the Malaria Pharmacognosy Laboratory in the Department of Pharmacology and Pharmacognosy in the Faculty of Pharmacy of University of Nairobi, Kenya. To prepare the water extracts, weighed amounts of plant material were put in conical flasks and boiled in water for 10-15 minutes, cooled and filtered using the suction pump or a funnel and cotton-wool. The filtrates were then stored at -20 0C and later freeze-dried or oven dried in small portions.

For ethnopharmacological screening of the claimed tocolytic and oxytocic properties of medicinal plants, water extracts were used. The fundamental logic behind using water as a solvent in the extraction was because the local people (traditional medical practitioners) administer most of their medicine in aqueous form. However, ethanol may be used as a solvent because at times traditional healers use locally made alcohol or fermented porridge or milk to extract and administer herbal drugs besides normal consumption of ethanol as a beverage by humans. In addition, in terms of polarity, ethanol is the safest substitute of water since it extracts most of the compounds that would be extracted by water.

**Procedure of preparing the rat uterus**

Young wistar virgin female rats weighing between 120-200g were used in the uterine motility experiments. The rats were obtained from the University of Nairobi, Faculty of Pharmacy animal house. The sensitivity of the uterus was increased by a subcutaneous injection of the abdomen with stilboestrol (0.1 mg/kg) in the laboratory of Clinical Pharmacology and Therapeutics in the Faculty of Medicine, University of Nairobi. The rats were left for 48 hours after which these young rats were sacrificed by a blow on the head. The uterus was carefully dissected in a petri dish of De Jalon Ringer Solution at 32 0C. The uterine horns separated from the animal just below the ovaries were cleaned and any extraneous fat and connective tissues were removed. The horns were then separated at the bifurcation, yielding two preparations. One preparation was taken and mounted in the aerated (95% O2 and 5 % CO2) organ bath at 32 0C to 37 0C, for 30 to 45 minutes to normalize before adding the plant crude extracts and standard drugs (oxytocin) so that the spontaneous activity could be deduced.

To minimise the experimental errors of dilution, the known weighed drugs were transferred into the test tubes and dissolved in de Jalons solution since 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, connected to the transducer and writing lever that was translating the tissue movements on the 7050 Microdynamometer
recording graph paper (Fig. 1). The tissue was always washed with De Jalon Ringer solution after every injected drug (or set of drugs) and recording was done before another drug (or set of 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 normalise before addition of another drug. The temperature of the organ bath containing the tissue was always maintained between 32-37°C.

Procedure of preparing the Rabbit Jejunum
A rabbit was sacrificed, the abdomen opened, the caecum was lifted forwards, and the ileum was found joined at the back of it. The duodenum was cut at a point 5 to 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 intestine and partly because of the Jejunum spontaneous activity that causes it to clear itself even after cutting it. Great care was taken to avoid damaging the gut muscle. It was handled with fingers rather than being gripped 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 see the jejunum is left open and then 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 was contracting rhythmically and regularly. After normalizing the tissue for about 30 minutes, the herbal drugs were introduced as well as standard drugs to act as controls and the pendular movements of the rabbit jejunum were translated by the transducer attached to the 7050 Microdynamometer Recording Machine on the 7050 Microdynamometer recording paper (Fig. 1.). The crude plant extracts of C. gynandra and V. amygdalina were screened for both oxytocic and tocolytic effects on the isolated rat uterus and rabbit jejunum and the motility of the tissue and time were recorded.

Figure 1. The 7050 Microdynamometer Recording Machine, recording of the uterine or jejunum motility
**Procedure of preparing the aqueous herbal drugs**

100 mg of aqueous crude plant sample were weighted using a weighing machine of up to 4 decimal places. The stock aqueous extracts for the bioassay were prepared by dissolving 100 mg of the crude extract in 5 ml of De Jalons solution for rat uterus or Tyrode’s solution for the rabbit jejunum experiments. The 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, one ml new disposable syringes were used. The tissues were mounted in the bathing solution of approximately 7 ml of De Jalons and Tyrode’s solutions for the uterus and jejunum tissues respectively. Therefore, the actual concentration of the herbal extract is that particular concentration drawn using the syringe plus the organ bath dilution effect. The concentration of the stock solution for all crude extracts was prepared by dissolving 100 mg in 5 ml of either De Jalons or Tyrode’s solution to obtain 20 mg/ml. Thus, 20 mg/ml is divided by 7 ml of the organ bathing solution to obtain the actual concentration used (Table 1).

<table>
<thead>
<tr>
<th>Concentration of the stock solution, (in ml)</th>
<th>Concentration, (mg/ml)</th>
<th>Final concentration in organ bath (mg/ml)</th>
</tr>
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<tr>
<td>0.1</td>
<td>2.0</td>
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<tr>
<td>0.2</td>
<td>4.0</td>
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<tr>
<td>0.3</td>
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<td>0.5</td>
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</tr>
<tr>
<td>1.0</td>
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<td>2.857</td>
</tr>
<tr>
<td>2.0</td>
<td>40.0</td>
<td>5.714</td>
</tr>
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</table>

**3. RESULTS**

**Ethnobotanical uses**

The roots and leaves decoction of *Vernonia amygdalina* are traditionally used in western Uganda to treat various ailments such as treatment of painful uterus, inducing uterine contractions, management of retained placenta and post partum bleeding, malaria, induced abortion, antimicrobes (bacterial and fungal infections), infertility, colic pains and treatment of irregular and painful menstruation (Kamatenesi-Mugisha 2004).

The roots of *Cleome gynandra* are chewed to induce uterine contractions and removal of retained placenta and control post partum bleeding in childbirth process. The roots, leaves and flowers of *Cleome gynandra* are used in the prevention of miscarriages and treatment of colic pains when boiled or cooked as food. The leaves, roots and flowers of *Cleome gynandra* are chewed, cooked or are sun-dried and drank in tea to treat sexual impotence or erectile dysfunctions in men (Kamatenesi-Mugisha, 2004; Kamatenesi-Mugisha & Oryem-Origa 2005).

*V. amygdalina* is mainly growing as a wild plant although in Cameroon is eaten as vegetable. *C. gynandra* is home grown and is widely used as an edible food and vegetable in most parts of Uganda and East Africa.
The effects of *V. amygdalina* on the uterine motility

The aqueous extract of *V. amygdalina* dissolved completely in the De Jalons solution. *V. amygdalina* extract, at 0.29 mg/ml, 1.43 mg/ml, 1.71 mg/ml and 2.86 mg/ml, showed increased rat uterine motility (Fig. 2 i, ii, iii). The extract of *V. amygdalina* displayed similar uterine motility as Oxytocin (Fig. 2, iii). With the increased concentration of *V. amygdalina*, uterine motility increased. Thus, the aqueous herbal extract of *V. amygdalina* causes uterine contractions.

(i) *V. amygdalina* (0.29, 1.71 mg/ml)  
(ii) *V. amygdalina* (1.43, 2.86 mg/ml)  
(iii) *V. amygdalina* (2.86 mg/ml) and Oxytocin (1 µg /ml)

**Figure 2.** The effects of *Vernonia amygdalina* on the uterine motility
The effects of *C. gynandra* on the uterine motility.

The aqueous root extract of *C. gynandra* (2.86 mg/ml) showed weak uterine motility (Fig. 3i) in comparison to the uterine motility caused by Oxytocin (Fig. 3ii). However, on combining *C. gynandra* (2.86 mg/ml) with Oxytocin (1.0 µg/ml) (Fig. 3 iii), the uterine motility increased slightly higher than Oxytocin alone but went down after 5 minutes. *Cleome gynandra* had weak uterine stimulating effects and might be acting to facilitate the activity of Oxytocin at childbirth.

(i) *C. gynandra* (2.86 mg/ml)  
(ii) Oxytocin (1.0 µg/ml)  
(iii) *C. gynandra* (2.86 mg/ml) + Oxytocin (1.0 µg/ml)

**Figure 3.** The effects of *Cleome gynandra* on the uterine motility
The effects of *V. amygdalina* on the jejunum motility

The different concentrations of *Vernonia amygdalina* (0.86, 1.43, 2.86 mg/ml) increased the jejunum motility (Fig. 4 i, ii). The stimulating effects of *V. amygdalina* (Fig. 4 ii) and the standard drug, Carbachol (Fig. 4 iii), showed similar behaviour by shifting the baseline and increased jejunum motility. The aqueous extract of *Vernonia amygdalina* contracted the smooth muscle of the rabbit jejunum and shifted the baseline upwards. The *V. amygdalina* (0.86 mg/ml) extract is able to cause effective jejunum contractions.

(i) *V. amygdalina* (0.86, 1.43, 2.86 mg/ml)  
(ii) *V. amygdalina* (2.86 mg/ml)  
(iii) Carbachol (2.0µg/ml)

**Figure 4.** The effects of *Vernonia amygdalina* on the jejunum motility
4. DISCUSSION
The rat uterus tissue was used in ethnopharmacological screening because *Vernonia amygdalina* and *Cleome gynandra* herbal remedies are used in stimulating childbirth traditionally. The smooth muscle of the rat uterus was the point of contact for these herbal drugs experimentation due to its high sensitivity among other laboratory animals. The aqueous extracts of *Vernonia amygdalina* and *Cleome gynandra* contracted and increased the uterine motility.

The aqueous herbal extract of *Vernonia amygdalina* of that showed marked rat uterine stimulation (oxytocic) was further re-screened using the smooth muscle of the rabbit jejunum to confirm the findings. The rabbit jejunum was used because of being a smooth muscle like the uterus and the pronounced pendular movements due to its high sensitivity. *Vernonia amygdalina* contracted the jejunum motility.

In the interpretation of the results, normal motility is the baseline for that particular tissue. Standard drugs were used as the controls of the set up. The medicinal plants selected for ethnopharmacological tests were based on the ethnobotanical indigenous knowledge. The fact that traditional healers have been using these plants for ages is a worthwhile reason to investigate their efficacy in the claimed use and matching preparations in the laboratory with indigenous knowledge.

The aqueous herbal extract of *V. amygdalina* is a strong oxytocic plant. The herbal extract was found to be long acting and when introduced to the rat uterus, it caused contractions that were sustained over 30 minutes. The decoction of *V. amygdalina* is used for treating malaria irrespective of age, gender, sex and pregnancy in western Uganda, yet the plant is oxytocic as displayed in (Figs. 2 and 4). The use of *V. amygdalina* extract to treat malaria in pregnancy is scientifically dangerous since it increased uterine motility. According to the findings in this work, the plant drug is oxytocic and may cause abortion when used in preterm pregnancy. The aqueous extract of *V. amygdalina* can cause uterine contractions at lower doses of less than 300 µg/ml. The usage of *V. amygdalina* to induce labour can, if due care is not taken; can cause uterine rupture or other complications to the mother and foetus. The aqueous herbal extract of *Vernonia amygdalina* caused the contraction of the smooth muscle of the rat uterus and rabbit jejunum. This is an indication that *V. amygdalina* can hasten childbirth or cause abortion if used in preterm pregnancy.

Ethnobotanical uses elsewhere show that the plant is widely used in Africa. In Malawi, the dried bark of *V. amygdalina* is used to improve uterine contractions during labour in pregnant women (Bullough, & Leary, 1982). However, the aqueous extract was found inactive on the guinea pig uterus (Bullough, & Leary, 1982). Lactating women who want to increase milk flow (Vasileva, 1969) drink hot water extract decoction. In Guinea-Bissau and Nigeria, the infusion of leaves of *V. amygdalina* is used as an abortifacient in women (Viera, 1959; Awe, et al., 1999). In Rwanda, the methanolic extract of *V. amygdalina* showed weak relaxant activity on the smooth muscle of the guinea pig ileum, but caused neither relaxation nor stimulation of the guinea pig uterine muscle (Chagnon, 1984). Although the ethnopharmacological experiments elsewhere where never proved more effective, it could have been due to the choice of the test animals used since the rat uterus in more sensitive than the pig uterine muscle.

The aqueous extract of *Cleome gynandra* showed weak stimulating effects on the uterus. On combining *C. gynandra* extract with Oxytocin, the motility increased slightly higher. The *C.
gynandra facilitated the activity of Oxytocin. The medicinal plants like Cleome gynandra, which is used all over Uganda to hasten childbirth, might be augmenting endogenous Oxytocin and may be useful in hastening the process of childbirth. Cleome gynandra is widely used in hastening childbirth (Oryem-Origa, et al., 2003).

Although herbal drugs are used in hastening childbirth, the doses used at term for initiating, sustaining, and augmentation of labour to hasten childbirth are very low. Childbirth being an emergency, only a few leaves are squeezed or a single root is chewed in traditional medicine. Thus, the usage of V. amygdalina in hastening childbirth is potentially safe particularly when administered by the traditional birth attendants with long standing experience in child births.

Unlike in the stimulation of abortion, the pregnant women consume large amounts of the plant extracts for some days either intentionally or while treating other diseases such as malaria to cause adverse effects. This would imply that the plant that has shown properties of contracting the uterus is hinged on the dose dependent ratio.

The plants that may cause mild effects when consumed such as Cleome gynandra to hasten labour and may not pose much serious threat in rupturing the uterine membranes. The plant species that have shown to augment Oxytocin such as Cleome gynandra on the isolated rat uterus could as well imply that they can augment the endogenous Oxytocin by sustaining the force and amplitude of contractions hence hastening childbirth.

The oxytocic plants such as V. amygdalina have the ability of hastening parturition. Exogenous oxytocin can initiate or enhance rhythmic contractions at anyone time. The uterine stimulating agents used most frequently to induce or augment labour in selectively pregnant woman (Dudley, 1997) is common in hospitals, as plants used by traditional birth attendants to hasten childbirth is also a common practice. However, considerable higher doses are required in early pregnancy for exogenous oxytocin to have action (Katzung, 1992; Dudley, 1997; Hardman, et al., 2001). This may also imply that use of oxytocic medicinal plant species such as V. amygdalina that double as antimalarial herbs in pregnancy may be safe depending on the dosage used, the stage of pregnancy and the vitality of the pregnant woman. Katzung (1992) reported that an eight-fold increase in uterine sensitivity to oxytocin occurs in the last half of pregnancy, although the use of exogenous oxytocin at any stage may facilitate labour progression. The use of exogenous oxytocics like plants in rural areas is important in cases where there is a significant arrest of dilation or descent though great care is required.

On comparison, the plant species such as Cleome gynandra with weak uterine motility when consumed in small quantities may help to hasten childbirth at the onset of labour and they may be less fatal than the definite oxytocic herbs where the dosage is not standardised as the case of conventional Oxytocin. However, further pharmacological tests to determine dosage are recommended for V. amygdalina and C. gynandra medicinal plants to warrant their safety.
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6. References


