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Chemical Composition and Oxytocic Properties of Traditional Herbal Medicines for Labour in Zambia: An HPLC-UV Analytical Study

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Abstract

Background: Traditional herbal medicines (THMs) are widely used during pregnancy and labour in Zambia, yet their chemical composition and safety remain poorly understood. Despite extensive ethnographic documentation, only ~1% of tropical species have undergone scientific screening, and pharmacological validation is lacking.

Purpose: This study aimed to analyze and characterize the chemical constituents of five THMs commonly used for labour in Mazabuka District, Zambia, with a focus on identifying oxytocin-like and misoprostol-like compounds to inform evidence-based maternal health policy.

Methods: Five THMs *Achyranthes aspera* (Mutobolo), *Azanza garckeana* (Mutobo), *Brassica oleracea* (Tende), *Cincenya* (Velvet fruit), and elephant dung—were collected and analyzed using High-Performance Liquid Chromatography with UV detection (HPLC-UV) at the National Food and Drug Control Laboratory, Lusaka, Zambia. Aqueous and methanolic extracts were compared against oxytocin (Rt = 12 min) and Cytotec/misoprostol (Rt = 7 min) reference standards.

Results: Three THMs Mutobolo, Mutobo, and Tende exhibited oxytocin-like activity, with Mutobolo showing dual peaks matching both oxytocin and Cytotec. Cincenya presented a distinct peak at 14 minutes, suggesting unidentified bioactive compounds, while elephant dung showed no detectable uterotonic activity.

Conclusion/ originality: Mutobolo demonstrates verified oxytocic and misoprostol-like activity, supporting its traditional use for labour induction and augmentation. However, lack of standardization and dosing control presents maternal safety risks. Further toxicological and clinical validation is required before integrating these THMs into formal maternal healthcare systems.

Keywords: Traditional Herbal Medicines, Oxytocin, Misoprostol, Achyranthes Aspera, HPLC-UV, Maternal Health, Zambia

1. Introduction

Medicinal plants represent an invaluable source of novel natural compounds offering chemical diversity essential to pharmaceutical innovation (Boligon & Athayde, 2014 [6]; Kumar *et al*., 2017). These botanicals serve as the foundation for complementary and alternative medicines, dietary supplements, cosmetics, and increasingly, medical devices (WHO, 2014; Muyumba *et al*., 2021) [30, 17]. However, quality control of herbal medicines presents substantial challenges for regulatory authorities, who must address issues ranging from identity verification to contamination assessment, risk identification, and legal compliance (Muyumba *et al*., 2021) [17]. The pharmaceutical quality of herbal medicines critically depends on harvest conditions, elapsed time between collection and analysis, and plant phenological stage (Muyumba *et al*., 2021) [17]. Storage conditions significantly influence microbiological quality, preservation of secondary metabolites, and overall therapeutic integrity (Sahoo & Manchikanti, 2010) [23]. Fresh samples are particularly fragile and prone to rapid deterioration. Poor storage and drying practices can result in spoilage, biological contamination, photo-decomposition, or atmospheric oxidation. Production-stage contamination further influences composition and quality of final products, with potentially significant impacts on chemical composition and therapeutic efficacy (Sahoo & Manchikanti, 2010; Muyumba *et al*., 2021) [23, 17].

Proper preparation of medicinal plants for experimental purposes constitutes an initial critical step in achieving quality research outcomes, involving systematic extraction and determination of bioactive constituent quality and quantity before biological testing (Abubakar & Haque, 2020) [1]. This chapter provides an introductory framework for understanding the chemical basis of traditional herbal medicines used during labour in Zambia. Although studies on traditional herbal medicines have proliferated in Zambia, comprehensive investigations focusing on chemical constituents remain notably absent from the literature. A substantial number of traditional medicinal species from previous ethnographic research, including those documented by Mwambula *et al*. (2019) [18] and recently by Siatwiko and Nkhata (2025) [24] in Mazabuka District, await chemical investigation. For tropical plant species, only approximately 1% have undergone scientific screening (Noureddine & Lahcen, 2024) [20].

Recent ethnographic research in Mazabuka District identified ten traditional herbal medicines commonly used during labour, revealing their deep cultural significance, specific preparation protocols, and perceived therapeutic applications (Siatwiko & Nkhata, 2025) [24]. Traditional birth attendants, community health workers, and herbalists reported using these remedies primarily for labour acceleration, management of spiritually attributed complications such as *Inchila* (obstructed labour linked to spousal infidelity), and birth canal preparation (Siatwiko & Nkhata, 2025) [24]. However, while this ethnographic study documented extensive utilization patterns and cultural beliefs surrounding these medicines, the pharmacological basis for their purported oxytocic effects remained scientifically unverified. Given the contradictory claims that traditional herbal medicines for labour possess beneficial pharmacological properties (Vicas & Mureş, 2020) [29] while simultaneously being characterized as potentially harmful (Alhassan *et al*., 2024; Makombe *et al*., 2023) [3, 15], urgent investigation of the chemical basis of these traditional medicines is essential. This study addresses the critical knowledge gap between documented traditional use and scientific validation, analyzing five of the ten previously identified medicines to determine their chemical composition and potential oxytocic properties, thereby generating evidence to inform safe integration into maternal healthcare systems.

2. Materials and Methods

2.1 Study Design and Framework

This analytical laboratory study investigated five traditional herbal medicines (THMs) commonly used for labour in Mazabuka District, Zambia: *Achyranthes aspera* (Mutobolo), *Azanza garckeana* (Mutobo), *Brassica oleracea* (Tende), *Cincenya* (Velvet fruit), and elephant dung. These were selected from ten remedies previously documented through ethnographic research (Siatwiko & Nkhata, 2025) [24]. The study aimed to identify oxytocin- and misoprostol-like compounds and characterize their chemical profiles using High-Performance Liquid Chromatography with UV detection (HPLC-UV), a method effective for analyzing complex plant extracts (Urbain & Simões-Pires, 2020; Al-Kaf *et al*., 2024) [28, 2]. The Evidence-Based Approach framework guided the study, aligning laboratory findings with policy-relevant maternal health insights (Dobuzinskis *et al*., 2005) [8].

2.2 Study Site

Mazabuka District, located in Southern Province, is a major agricultural zone and commercial hub. It lies 125 km south of Lusaka and had a population of 234,045 in 2022, with women comprising 51.2% (CSO, 2022) [7]. High maternal mortality rates (14% of provincial deaths in 2023) and cultural reliance on THMs made Mazabuka a strategic site for this investigation (Siatwiko & Nkhata, 2025) [24].

2.3 Sample Selection and Preparation

Five THMs were randomly selected from ten previously identified remedies. Plant roots were harvested, cleaned, and pounded; elephant dung was processed in powdered form. All samples were dried, coded, and transported to Lusaka for analysis at the National Food and Drug Control Laboratory due to lack of HPLC facilities in Mazabuka.

2.4 HPLC-UV Analysis

Powdered samples were extracted in water and methanol (100 g/1000 mL), agitated for 48 hours, filtered, and concentrated at 40°C. Chromatographic separation used a gradient mobile phase of distilled water and acetonitrile, both with 0.1% trifluoroacetic acid. Instrument settings followed USP standards: flow rate 1.0 mL/min, gradient 5–50% over 30 minutes, detection at 220 nm, injection volume 10 µL, and run time 45 minutes (USP, 2025) [27]. Oxytocin and Cytotec (misoprostol) standards were injected separately, with retention times of 12 and 7 minutes, respectively. These benchmarks were used to identify uterotonic compounds in THM extracts (Rashed, 2024) [21].

2.5 Data Collection and Analysis

To ensure unbiased selection, all ten THMs were coded and blinded. Five were randomly chosen for analysis. Sample preparation followed USP protocols to ensure methodological rigor. HPLC-UV was used to detect uterotonic activity by comparing retention times with reference standards. Peaks matching oxytocin or Cytotec indicated pharmacological similarity. This method was selected for its sensitivity and reliability in natural product analysis (Urbain & Simões-Pires, 2020; Al-Kaf *et al*., 2024) [28, 2].

2.6 Ethical Considerations

Ethical approval was granted by the University of Lusaka IRB (Ref: FWA00033228-00403/25) and the National Health Research Authority (Ref: NHRA-2107/04/04/2025), with additional clearance from Mazabuka District Health Office and UTH Laboratory. Informed consent was obtained from all participants. Confidentiality was maintained through anonymization and coded samples. Results were shared in culturally appropriate formats, respecting intellectual property and community ownership of traditional knowledge.

3. Results

3.1 Overview of Uterotonic Activity

High-Performance Liquid Chromatography with UV detection (HPLC-UV) revealed oxytocin-like activity in three of the five traditional herbal medicines (THMs) tested. Retention times were compared against pharmaceutical standards: oxytocin (12 minutes) and Cytotec/misoprostol (7 minutes). Mutobolo (*Achyranthes aspera*) showed dual activity, while Mutobo (*Azanza garckeana*) and Tende (*Brassica oleracea*) exhibited mild oxytocin-like effects. Cincenya (Velvet fruit) and elephant dung showed no standard uterotonic peaks, though Cincenya presented a distinct peak at 14 minutes.

3.2 Interpretation of Table 1: Chemical Composition and Retention Times

Table 1 summarizes the retention times, presence of oxytocin and Cytotec-like compounds, and key pharmacological observations for each THM. It provides a concise comparison of chemical activity across the five samples. This table supports the narrative findings and provides a reference point for interpreting chromatographic data.

Table 1: Chemical Constituents and Uterotonic Activity of Traditional Herbal Medicines

THM Name	Oxytocin Activity	Cytotec Activity	Retention Time (min)	Key Observations
Mutobolo (*Achyranthes aspera*)	High	High	12 & 7	Dual activity; potent uterotonic effects; extractable in water and methanol
Mutobo (*Azanza garckeana*)	Mild	None	12	Gentle stimulation; suitable for second-line use
Tende (*Brassica oleracea*)	Mild	None	12	Mild effect; antioxidant-rich; appropriate for gradual labour progression
Cincenya (Velvet Fruit)	None	None	14 (sharp peak)	Unidentified compounds; potential alternative mechanisms; safety concerns
Elephant Dung	None	None	—	No detectable activity; traditional efficacy may involve non-pharmacological factors

3.3 Interpretation of Fig 1: Oxytocin-Like Activity Comparison

Fig 1 illustrates the relative oxytocin-like activity of each THM, benchmarked against the pharmaceutical oxytocin standard. Mutobolo demonstrates full activity (100%), Mutobo approximately 40%, and Tende around 20%. Cincenya and elephant dung show no detectable oxytocin-like response. This figure visually reinforces the potency hierarchy among the tested remedies and supports the ethnographic classification of Mutobolo as a primary intervention.

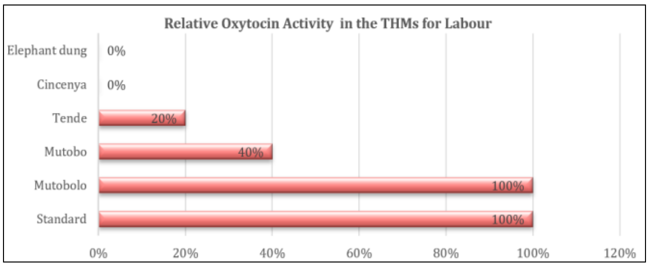

Fig 1: Oxytocin-Like Activity of THMs

3.4 Interpretation of Fig 2: Cytotec-Like Activity Comparison

Fig 2 presents Cytotec-like activity across the THMs. Only Mutobolo exhibited a peak at 7 minutes, indicating misoprostol-like properties. This dual activity suggests its potential for both labour induction and augmentation. This figure highlights Mutobolo's unique pharmacological profile and supports its prioritization in traditional labour protocols.

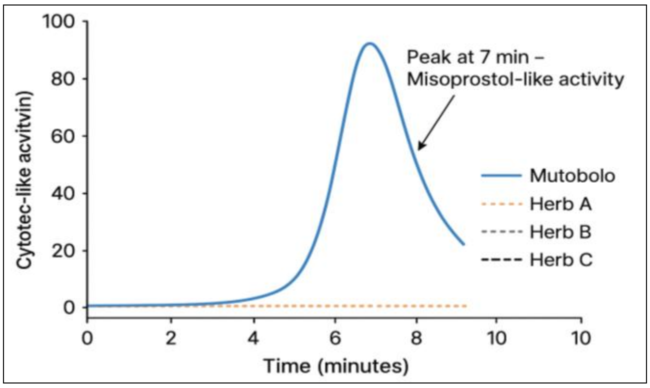

Fig 2: Cytotec-Like Activity of THMs

3.5 Additional Observations and Supplementary Analysis

Further HPLC-UV analysis revealed additional peaks, particularly in Mutobolo, suggesting the presence of flavonoids, phenolic compounds, minerals, or contaminants (Muyumba *et al*., 2021) [17]. Paper chromatography identified ergometrine-like alkaloids in Cincenya and quinine-like alkaloids in elephant dung respectively which may explain traditional efficacy but raises safety concerns due to risks of uterine hyper stimulation and vascular complications.

4. Discussion

This study provides the first chemical validation of traditional herbal medicines (THMs) used for labour in Zambia. HPLC-UV analysis confirmed oxytocin-like and misoprostol-like activity in *Achyranthes aspera* (Mutobolo), with milder effects in *Azanza garckeana* (Mutobo) and *Brassica oleracea* (Tende). These findings align with ethnographic data from Mazabuka District, where traditional birth attendants and herbalists use these remedies in a tiered protocol based on labour stage and intensity (Siatwiko & Nkhata, 2025) [24]. The dual activity of Mutobolo supports its documented use for both labour induction and augmentation. Its extractability in both water and methanol validates traditional water-based preparation methods. The alignment between chemical findings and traditional treatment hierarchies Mutobolo as first-line, Mutobo and Tende as gentler alternatives demonstrates empirical pharmacological reasoning within traditional systems.

Cincenya (Velvet fruit) and elephant dung showed no standard oxytocin or Cytotec-like activity, yet remain widely used. Cincenya's sharp peak at 14 minutes and detection of ergometrine-like compounds suggest alternative uterotonic pathways, possibly involving serotonin or adrenergic receptors (Ron *et al*., 2000) [22]. Ritual significance, placebo effects, and synergistic compound interactions may also contribute to perceived efficacy (Talebi *et al*., 2020) [26]. This pharmacological paradox where traditional efficacy is reported despite lack of detectable oxytocic compounds may also reflect methodological limitations. Similar findings have been reported in other contexts, such as the use of boiled dill seeds to shorten labour without conventional uterotonic markers (Talebi *et al*., 2020) [26].

The potent activity of Mutobolo raises risks of uterine hyper stimulation, especially when combined with pharmaceutical oxytocin. Ergometrine-like compounds in Cincenya pose risks of sustained contractions and hypertensive episodes. Elephant dung introduces microbiological hazards, including exposure to pathogens (Muyumba *et al*., 2021) [17]. Unlike pharmaceutical oxytocin, THMs are administered without standardized dosing, monitoring, or regulation which further causes compounds safety risks and can lead to uterine hyper stimulation, fetal distress, or postpartum complications (Makombe *et al*., 2023; Kaingu *et al*., 2011) [15, 11].

Women often conceal THM use from healthcare providers due to stigma and fear of judgment, limiting clinicians' ability to manage labour safely (Siatwiko & Nkhata, 2025) [24]. Yet, both traditional and biomedical practitioners express interest in collaboration, recognizing the value of each system. Successful models from Ghana and South Africa show that respectful integration can improve outcomes (Aziato & Omenyo, 2018; Ngomane & Mulaudzi, 2012) [4, 19]. Current policy approaches either prohibit or ignore THM use, leaving women unprotected. There is an urgent need for evidence-based regulation, practitioner training, and pharmacovigilance systems. Regionally, the identification of oxytocin-like activity in Zambian THMs parallels findings from Kenya, Tanzania, and Malawi (Kaingu *et al*., 2011; Haruna *et al*., 2017; Makombe *et al*., 2023) [11, 9, 15]. However, unique cultural practices in Mazabuka such as the use of elephant dung and the concept of Inchila underline the importance of localized research and culturally sensitive policy development.

5. Implications

The validation of Mutobolo's dual activity confirms its therapeutic potential but raises serious safety concerns. Uncontrolled use especially alongside hospital-administered oxytocin can lead to uterine hyper stimulation, fetal distress, or postpartum complications. The detection of ergometrine-like compounds in Cincenya complicates its safety profile, as ergot alkaloids can cause sustained contractions and hypertensive episodes during active labour (Ron *et al*., 2000) [22]. Additionally, elephant dung introduces microbiological risks, including exposure to pathogens and contaminants (Muyumba *et al*., 2021) [17].

Ethnographic findings reveal that women's concealment of THM use stems from lack of culturally competent care. Structured communication and mutual respect are essential for integrated care models that prioritize maternal and neonatal safety. While traditional knowledge systems have developed empirically effective protocols, the absence of regulation, standardized dosing, and safety monitoring presents serious risks. Integration into formal healthcare requires scientific validation, toxicological assessment, and culturally respectful collaboration between traditional and biomedical practitioners.

6. Conclusion

This study confirms the pharmacological basis of several traditional remedies used for labour in Zambia. Mutobolo demonstrates potent dual activity, validating its role in both induction and augmentation. Mutobo and Tende offer gentler stimulation, supporting their use in earlier labour stages. The absence of standard uterotonic activity in Cincenya and elephant dung highlights the complexity of traditional medicine systems and the need for further investigation. Integration into formal healthcare demands scientific validation, regulation, and culturally sensitive collaboration.

7. Recommendations

Healthcare providers should encourage open disclosure of THM use during antenatal and labour care through non-judgmental communication. Training programs must equip providers with culturally competent care skills and integrate routine screening for THM use into maternal health records. Given the safety concerns identified in this study, the use of Cincenya and elephant dung should be discouraged until their safety profiles are fully understood through rigorous toxicological assessment. Further research is essential to determine the chemical composition, appropriate dosing, and toxicity profiles of THMs using advanced analytical and bioassay techniques. This scientific validation must be accompanied by the development of national policy and regulatory frameworks to guide the safe integration of validated THMs into Zambia's maternal healthcare system. Such frameworks should balance respect for traditional knowledge with evidence-based safety standards, ensuring that women receive care that is both culturally appropriate and clinically safe.

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10. Conflict of Interest

The authors declare no conflicts of interest, financial or otherwise, related to this research.

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