African Journal of Pharmaceutical Research and Development
Available online at https://ajopred.com
Vol. 17 No.1; pp. 9-17 (2025)

Original Research Article

UTEROTONIC EFFECT OF METHANOL ROOT BARK EXTRACT OF *ENTADA MANNII* (OLIV.) TISSER

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ABSTRACT

Entada mannii (Oliv.) Tisser. (Fabaceae) has a range of uses in African traditional medicine such as treatment of respiratory ailments, gonorrhoea, yellow fever and toothache. This study is aimed at justifying folkloric usage of *E. mannii* in inducing labour, and it evaluated possible contractile effect of the crude methanol (MeOH) extract and aqueous (AQ) fraction of the root bark on isolated uterus of oestrogenised non-pregnant female rats. Spontaneous contraction model at 1.0 - 8.0 mg/mL extract, oxytocin-induced contraction model at 6.7 µg/mL in physiological salt solution (PSS), calcium ions-free PSS model, and high KCL concentration-induced contraction (80 mM) model were employed. Extract gave higher increases in amplitude (2-fold) and frequency (> 2 fold) of spontaneous uterine contraction than AQ fraction in a dose-dependent manner. For the oxytocin pre-contracted uteri in normal PSS and calcium ions free-PSS, as well as high KCL concentration-induced contraction, decreases in amplitude and frequency of contractions were recorded with both agents, suggesting the plant did not potentiate the effects of these agonists. This result, suggests smooth muscle uterotonic effect for *E. mannii* root bark.

ARTICLE INFO

Received 25 August, 2024
Accepted 07 June, 2025
Published 30 June, 2025

KEYWORDS
Entada mannii,
Root bark,
Uterotonic effect,
Rat uterus,
Methanol extract,
Aqueous fraction

INTRODUCTION

Entada mannii (Oliv.) Tisser. (Fabaceae), is commonly known as African dream herb and locally as 'ogurobe' in Yoruba. It is a scandent shrub up to 30 m in height growing on rocky hills in the forest from Senegal to Zaire and Angola [1]. The bark is brown-grey to black with a very rough, transversely stripped, scaly, with a peeling in long fibrous strips which has a red or yellow-brown slash fibrous. The ethnomedicinal uses, phytochemistry and pharmacology of the more common related species, *E. africana* are well documented in literature [2]. Apart from toxicological [3] and cardiac bio-tolerance [4] activities, extensive pharmacological and phytochemical investigations of *E. mannii* are unknown, and no bioactive compounds have been reported. However, bioactive compounds such as flavonoids, terpenoids and a phenolic compound with antibacterial, cytotoxic and antioxidant activities from related species, *E. abyssinica* [5], as well as terpenoids, saponins and flavonoids from *E. africana* [2] have been reported.

General reviews of literature on medicinal plants used to induce labour during pregnancy in Sub-Saharan Africa [6,7], and globally [8] which excluded *E. mannii* have been published. Specifically, uterine contractility of nine medicinal

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https://doi.org/10.59493/ajopred/2025.1.2 ISSN: 0794-800X (print); 1596-2431 (online)

plants used to facilitate childbirth in Nigeria has been documented [9]. According to El Hajj and Holst [7], Nigeria ranks among the top countries in West Africa with high prevalence of oxytocic herbal medicines in pregnancy. Recently, the uterotonic effect of *Psidium guajava* stem bark [10] methanol extract and fractions, and Trema orientale root bark [11] were published.

Based on the information from traditional medical practitioners, *E. mannii* has been used in Nigerian traditional medicine to facilitate the induction of labour in pregnant women, but its scientific basis has never been reported. As part of the ongoing research thrust in our laboratory to update the database of folkloric childbirth inducers, *we* therefore investigated the methanol extract and aqueous fraction of the root bark of this folkloric labour inducers, we therefore investigated the methanol extract and aqueous fraction of the root bark of *E. mannii* for possible effect on non-pregnant female rats and herein report our findings.

MATERIALS AND METHODS

Plant collection and extraction

Root bark peels of *E. mannii* were harvested from the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria, in January 2022, and authenticated (voucher no IUO/22/403) at the herbarium of the Department of Pharmacognosy, Igbinedion University Okada (IUO). The bark peels were cut into small pieces and air-dried for seven days on a concrete floor, ground into coarse powder using a locally fabricated grinder. The powdered plant (350 g) was exhaustively extracted by cold maceration with 5L MeOH. The mixture was filtered, and the filtrate concentrated by open evaporation on an electric water bath at 40 °C to yield the crude extract. Part of the extract was re-dissolved in water and partitioned exhaustively with dichloromethane (DCM) in a separating funnel to yield aqueous (AQ) and DCM fractions. Fractions were concentrated to dryness and respective yields recorded. Dried extract and fractions were refrigerated at 4^{o}C until needed.

Drugs and reagents

The drugs for this study included stilboesterol and oxytocin purchased from Sigma-Aldrich, USA whole reagents such as NaCl, $NaClO_3$, KCL, $CaCl_2$, $MgCl_2$, $NaHCO_3$ and D-glucose were sourced from BDH Chemicals, England. Analar grade methanol was procured from BDH Chemicals, England.

Preparation of physiological salt solution

The biological assay was carried out in Pharmacology laboratory, University of Benin, Edo State, Nigeria. Firstly, the physiological salt solution (PSS) was prepared by dissolving appropriate amounts of NaCl, $NaClO_3$, D-glucose and KCL in appropriate amounts in distilled water in a calibrated beaker [12]. This was followed by $CaCl_2$ to avoid cloudiness to form the PSS with final composition in mM/L: NaCl 154.00, $NaHCO_3$ 5.95, D-glucose 2.78, KCl 5.63, and $CaCl_2{\cdot}2H_2O$ 2.05.

Experimental animals

Healthy non-pregnant albino rats (150 - 180 g) were utilized for this study. They were housed under standard conditions (27±5 °C and natural light and dark cycles) in the Central Animal House, University of Benin, and fed with standard animal pellets (Bendel Foods and Flower Meal, Edo State, Nigeria) and water *ad libitum.* Animals were handled following the Public Health Service policy on humane care and use of Laboratory Animals [13]. Ethical permission (IUO/ETHICS/024) was obtained before the start of the experiments from the Animal Ethics Committee, College of Pharmacy, IUO, Nigeria.

Preparation of uterine tissue

Female rats previously primed with stilboesterol (1 mg/kg) for 24 h were used for the study as described [12]. Animals were humanely killed by cervical dislocation and the uterine horns were immediately removed and placed in a petri dish containing previously warmed and aerated PSS. Connective and adhering tissues were removed from the isolated uterus, and one horn was dissected in half to obtain a segment of the uterine horn of approximately 1–2 mm in length. The oestrogenized uterine segment obtained was mounted in a warmed tissue organ bath (10 mL) maintained at 37°C and containing aerated PSS. The organ bath was connected to an isometric force transducer (7003E- Ugo Basile, Varese, Italy) linked to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy). The tissue was equilibrated under resting tensions of 4.90 mN for 30–45 min or till regular contractions were obtained.

Determination of uterine contractility

The MeOH extract and AQ fraction, at doses of 1.0 - 8.0 mg/mL, based on routine experimentations in our laboratory after acute toxicity test, were added separately to the organ bath containing isolated oestrogenized uterine tissue suspended in PSS to obtain concentration-response relationships [12]. Each concentration was allowed a contact time of 5 min before measuring the amplitude and frequency of contraction. Effect of extract and fraction on oxytocin-induced uterine contraction was similarly determined by adding 10 mg/mL to the tissue pre-contracted with 6.7 µg/mL oxytocin. Furthermore, samples (10 mg/mL) were tested on isolated uterus pre-contracted with high KCl concentration (80 mM/mL).

Determination of effect of extract in Ca^{2+}-free medium

In this experiment performed as previously described [12], PSS devoid of calcium was used, and ethylenediaminetetraacetic acid (EDTA) was substituted. Oestrogenized uterine tissue was initially equilibrated for 30 min with former PSS and replaced with EDTA (1mM). Tissue was then equilibrated in this Ca^{2+}-free solution for 3–5 min (it was essential that contractions were not totally diminished during the experiment to allow for measurements). After equilibration, oxytocin (6.7 µg/mL) was added, and a contact

time of 5 min was allowed. Without flushing, extract (10 mg/mL) was added, and a contact time of 5 min was allowed before measuring the amplitude of contraction.

RESULTS

Effect on spontaneous uterine contraction

When rat uterus was treated with crude MeOH extract and AQ fraction *in vitro,* both amplitude and frequency increased dose-dependently. Amplitude value attained a peak of 2.133 gr at sub-optimal dose, 4 mg/ml for extract and 1.210 gr for fraction at the highest concentration (8 mg/mL) (Figure 1A-1D). It then decreased slightly to 2.047 gr (4.03% decrease) with extract only. Interestingly, frequency data followed the same pattern as amplitude for both tested agents, increasing from an initial 32 peaks/min to a maximum of 46 peaks/min (43.74% increase) at suboptimal dose for the extract and then a sharp decline to 34 peaks/min at optimal dose. However, frequency peaked at 23 peaks/min at the highest dose for AQ fraction (Figure 1C).

Effect on oxytocin-pretreated uterine contraction and calcium-free medium

Extract and AQ fraction tested at 10 mg/ml gave 16.66% and 25% inhibitions, respectively in contraction amplitude when uterus was pretreated with oxytocin (6.7 µg/ml) in normal salt medium (Figures 2A-2D). However, only the extract inhibited frequency of contraction by 25%. In a similar manner, exclusion of Ca^{2+} from the PSS also led to inhibition of oxytocin by both extract (25% decrease in amplitude) and AQ fraction (16.55% decrease) tested at 10 mg/ml (Figures 3A-3C).

Effect of high KCl-induced contraction

Extract, at 10 mg/ml suppressed amplitude of contraction by 25% in the presence of high KCL concentration (80 mM) (Figures 4A, 4C), while AQ fraction gave slight potentiation (2.53%) (Figures 4B, 4C), and hence appears to be more active.

DISCUSSION

From the spontaneous contraction experiment in this study, it is reasonable to ascribe uterotonic activity to the methanol extract and AQ fraction of *E. mannii* root bark. In comparing both tested agents, crude extract which gave greater force of contraction (2-fold) in both amplitude and frequency, can be adjudged to be more active. Results obtained with the two tested agents in this study indicate that they acted on the uterine smooth muscle of female rats via a similar mechanism as oxytocin **[**8]. Mechanism of uterine contractility of oxytocin is reported to be via myometrial oxytocin receptors and on endometrial oxytocin receptors to stimulate prostaglandins and cholinergic releases, as well as by elevating intracellular calcium concentration [8]. **The** mechanisms of spontaneous contraction of uterine smooth muscle have been postulated to proceed via inhibition of K^+ channels and sarcoplasmic reticulum Ca^{2+} ATPase, and to release of prostaglandins, respectively [8. 14].

The response of the AQ fraction to uterus pretreated with oxytocin suggested potentiation with reference to frequency (Figure 2D), and hence more active than the MeOH extract. It could be inferred further that tested agents possibly acted by a different mechanism compared with the standard agonist, oxytocin. Inhibition of contraction amplitude by both tested agents in PSS devoid of Ca^{2+} is a pointer to the possible irrelevance of these ions in the contraction of uterine smooth muscle. This is in consonance with the reports of other workers [16,17]. Inhibition of oxytocin in Ca^{2+} deficient PSS was considered as evidence of non-specific uterine relaxant activity [18]. Among the six plant components in the traditional oxytocic herbal recipe in Southeast Nigeria, five plants, *Barteria fistulosa, Napoleonaea vogelii, Spondias mombin, Euphorbia convolvuloides* and *Ceiba pentandra,* were found to exhibit varying degrees of inhibition of L-type Ca^{2+} channels and sarcoplasmic reticulum Ca^{2+} release [19]. Similarly, *Ficus capensis* [20] and *Justicia flava* [12] among others, have been reported to relax rat uterine smooth muscle pre-contracted with oxytocin *in vitro.* According to present study, while mechanism of action of *E. mannii* crude extract appears contrary to that of KCl, AQ fraction possibly exhibited similar mechanisms as KCl. Moreso, inhibition of uterine contractility has been suggested to involve blockade of extracellular and intracellular calcium entry [12], as well as occurring at agonist receptor level or through the parasympathetic (cholinergic) nervous pathway [18].

The inhibitory effects on the rat's uterine smooth muscle tissue has been attributed to some bioactive compounds such as palmitic and oleic acids [19]. However, a review of uterine relaxant effects of plants and isolated bioactive compounds predominantly flavonoids and terpenes have been published [16]. Phytometabolites of *E. mannii* such as flavonoids, sterols, polyterpenes, polyphenols, alkaloids and saponins as previously reported [3] could be responsible for the observed effects on uterine smooth muscle. Hence, the MeOH root bark extract of *E. mannii* investigated herein, which has demonstrated potential as an uterotonic agent can be considered relevant in this regard

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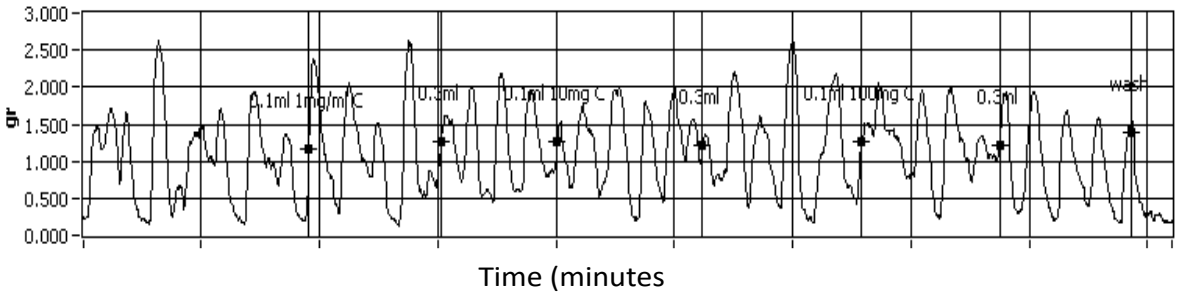


Figure IA: Chart of methanol extract of *Entada mannii* (EM) stem bark on spontaneous contraction of non-pregnant rat uterus

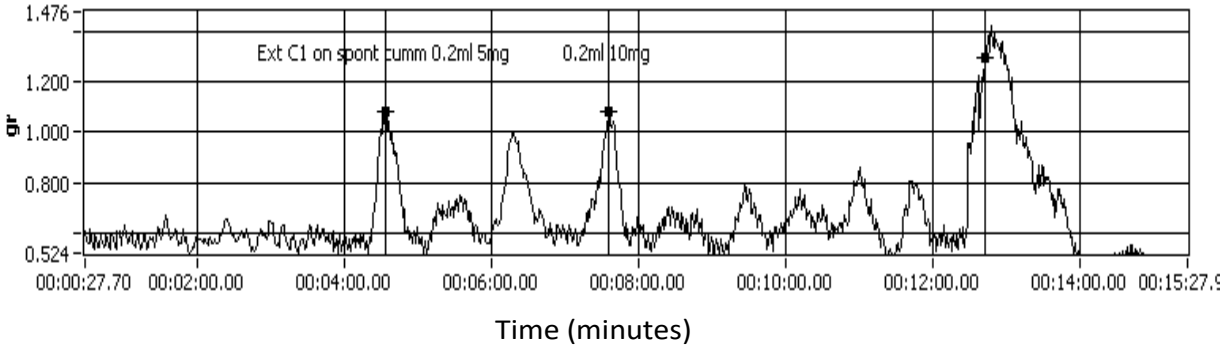


Figure IB: Chart of aqueous fraction of EM stem bark on spontaneous contraction of non-pregnant rat terus

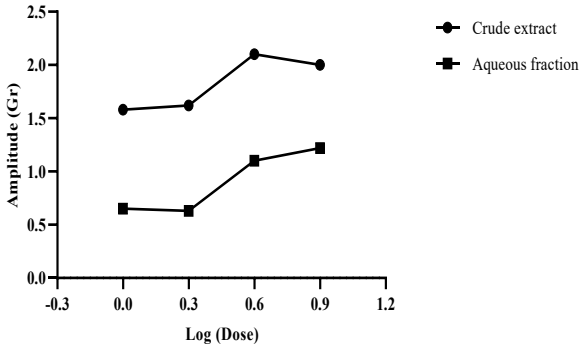


Figure 1C: Effect of EM stem bark crude extract and AQ fraction on amplitude of spontaneous contraction

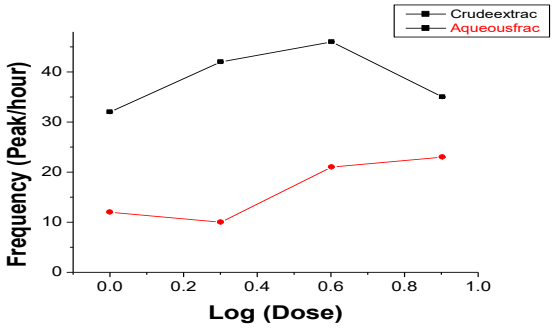


Figure 1D: Effect of EM stem bark crude extract and AQ fraction on frequency of spontaneous contraction

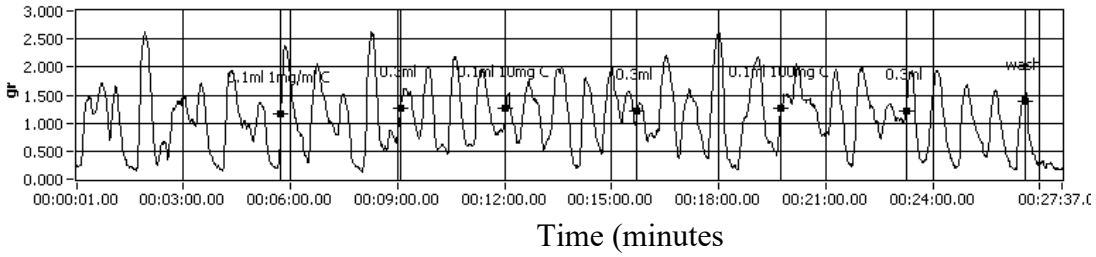


Figure IA: Chart of methanol extract of *Entada mannii* (EM) stem bark on spontaneous contraction of non-pregnant rat uterus

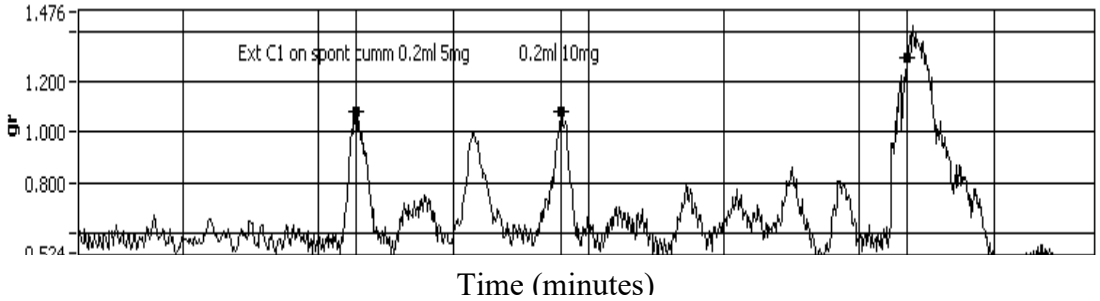


Figure IB: Chart of aqueous fraction of EM stem bark on spontaneous contraction of non-pregnant rat uterus

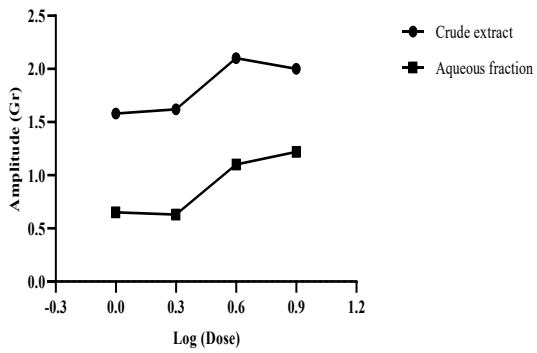


Figure 1C: Effect of EM stem bark crude extract and AQ fraction on amplitude of spontaneous

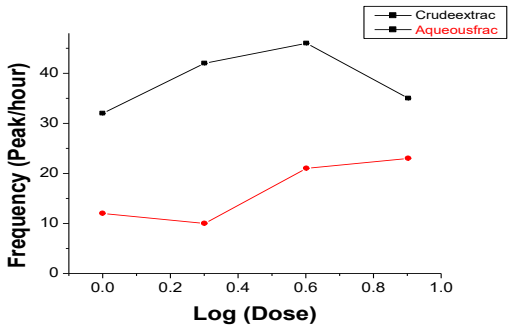
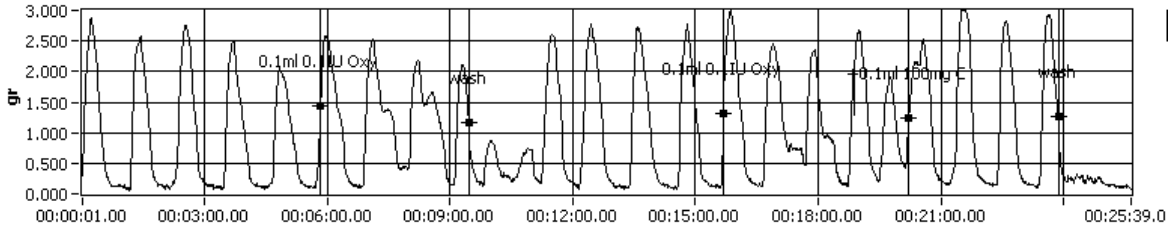
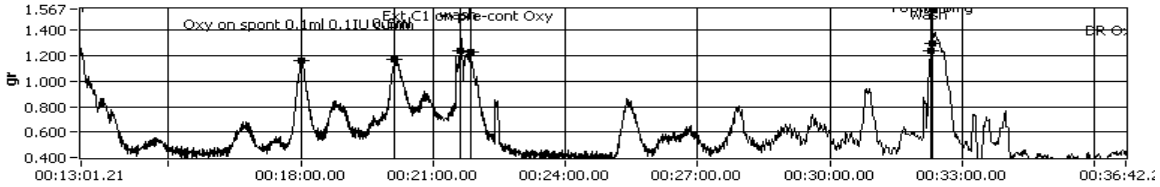


Figure 1D: Effect of EM stem bark crude extract and AQ fraction on frequency of spontaneous contraction



Time (minutes)

Figure 2A: Recording chart of crude MeOH extract of EM on oxytocin pre-contracted uterus in normal medium



Time (minutes)

Figure 2B: Recording chart of aqueous fraction of EM on oxytocin pre-contracted uterus in normal medium

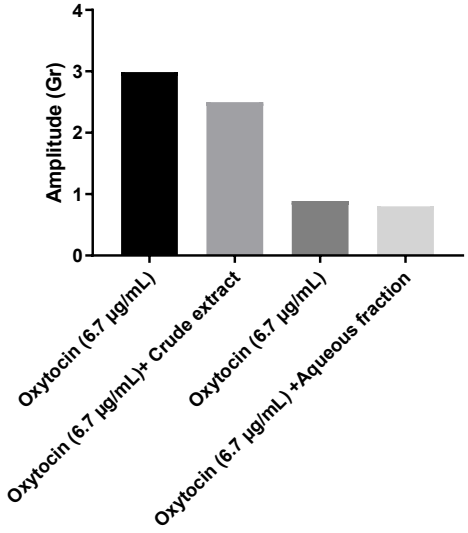


Figure 2C: Effect of EM on amplitude in oxytocin pre-contracted uterus in normal medium

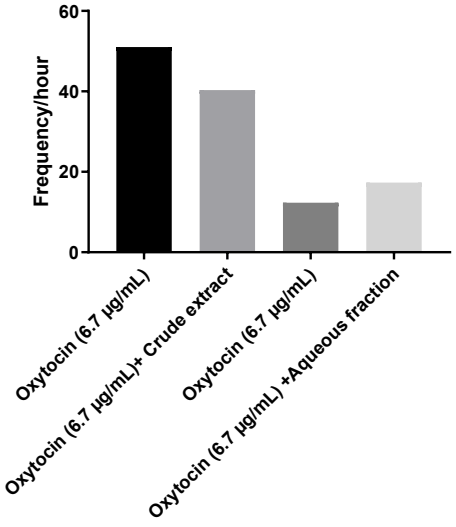
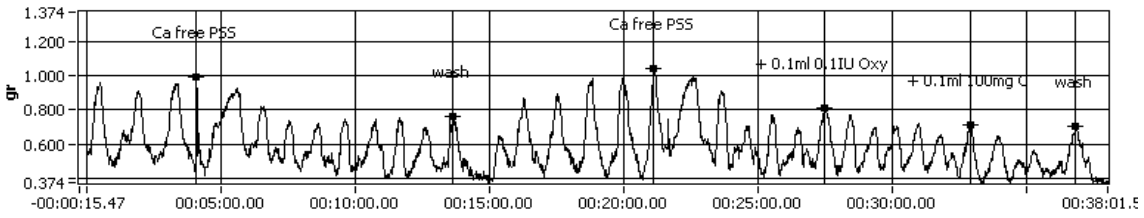
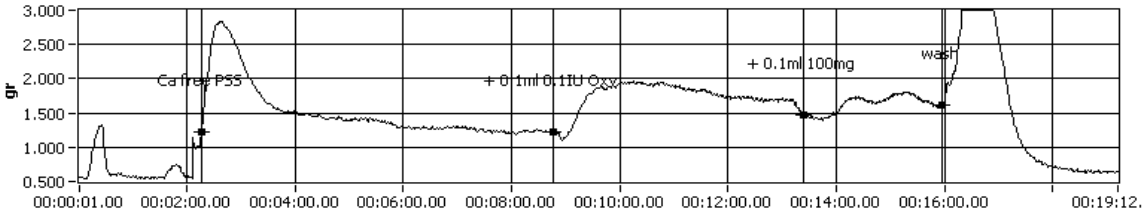


Figure 2D: Effect of EM on frequency in oxytocin pre-contracted uterus in normal medium



Time (minutes)

Figure 3A: Recording chart of methanol extract of *Entada mannii* (EM) on oxytocin pre-contracted uterus in calcium-free medium



Time (minutes)

Figure 3B: Recording chart of aqueous fraction of EM on oxytocin pre-contracted uterus in calcium-free medium

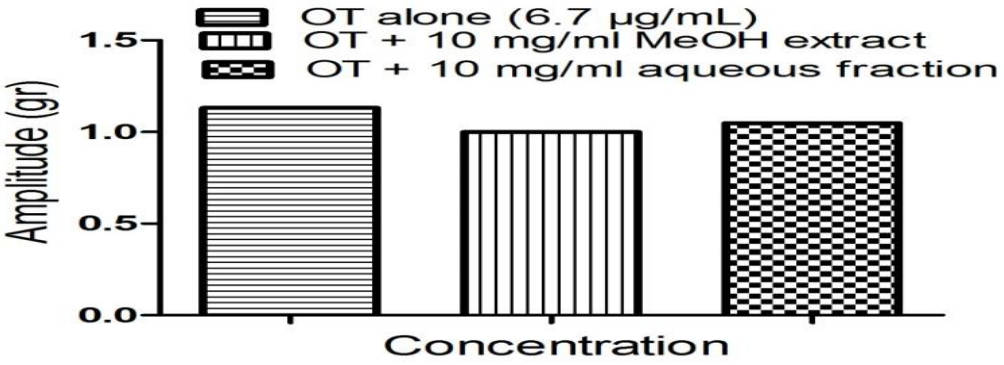
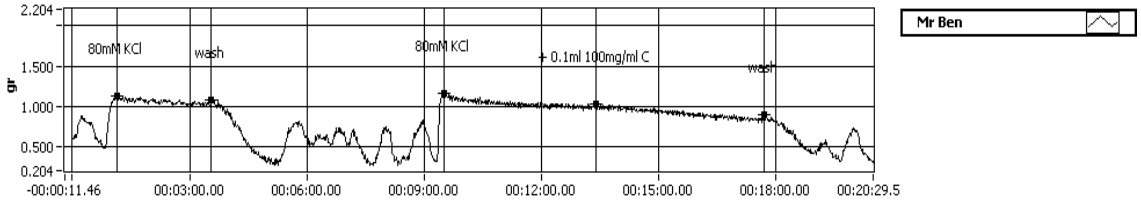


Figure 3C: Effect of EM on amplitude in oxytocin pre-contracted uterus in calcium-free medium



Time (minutes)

Figure 4A: Recording chart of crude MeOH extract of *Entada mannii* (EM) on high KCL concentration-pretreated uterus in normal medium

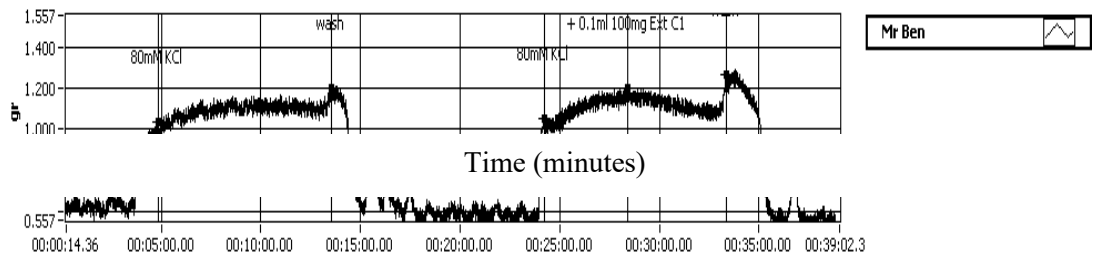


Figure 4B: Recording chart of EM aqueous fraction on high KCL concentration-pretreated uterus in normal medium

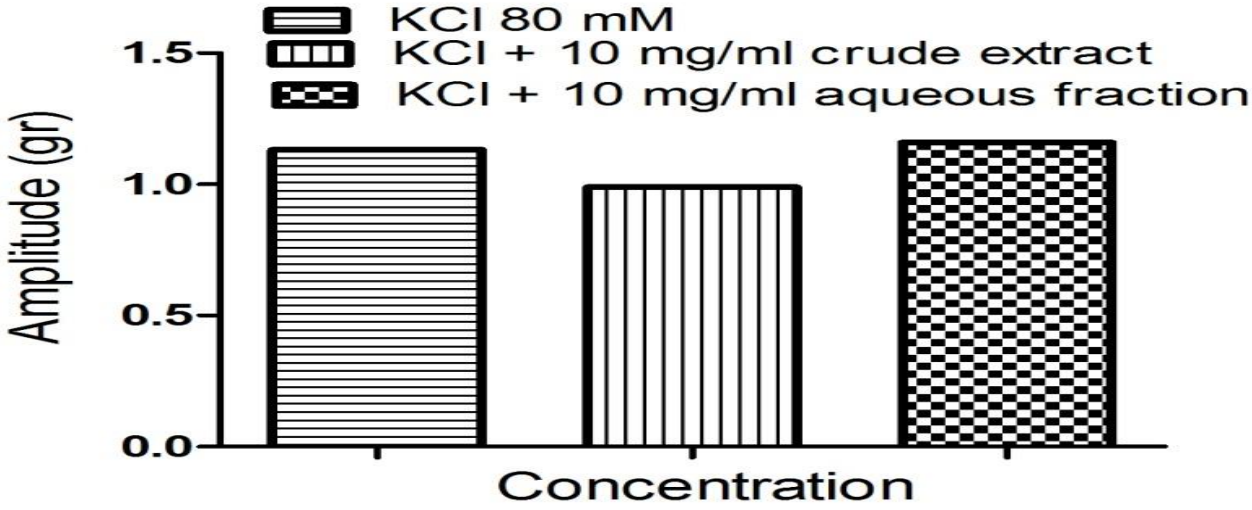


Figure 4C: Effect of EM tested agents on amplitude in high KCL concentration-pretreated uterus in normal medium

CONCLUSION

Current investigation represents the first scientific report of *E. mannii* as an uterotonic agent with potential usefulness in childbirth delivery. Further pharmacological and phytochemical investigations need to be performed on the plant to ascertain its safety profile, identify the bioactive constituents and confirm the possible mechanism of action.

ACKNOWLEDGMENT

Authors deeply appreciate Dr. O. A. Adedokun of Pharmacognosy Department for assistance with assay procedures and statistical analyses in the course of this work. Staff in Pharmacognosy laboratory, College of Pharmacy, Igbinedion University Okada, as well as staff of Department of Pharmacology and Animal house, University of Benin, are acknowledged for their technical contribution.

AUTHORS' CONTRIBUTION

Adebayo Anthony Gbolade: conceived the idea of the research, planning, co-execution of the research, and solely wrote the manuscript. Sonia Kesiena Ogona: participated in the bench work

CONFLICT OF INTEREST

Authors have declared that no competing interests exist in this work.

FUNDING

This research work was purely funded from personal emoluments of the authors. No funding whatsoever was received from local or external funding agencies.

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