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(RESEARCH ARTICLE)

In vitro uterotonic activities of cold aqueous stem bark extract of *Erythrophleum suaveolens* on estrogenized uterus of Nulliparous Guinea Pig

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Abstract

Medicinal plants have been used for different purposes. Interests in pharmacological and other scientific screening for the authentication of folkloric claims and drug development has become imperative. This study was aimed at investigating the effect of cold aqueous crude stem-bark extract of *Erythrophleum suaveolens* (CASBEEs) on estrogenized uterus of nonpregnant Guinea pig. Stem-bark of *E. suaveolens* were harvested, gently rinsed with clean water and dried under shade in a well-ventilated environment, ground into powder and cold aqueous crude extract of the processed plant material was obtained via maceration. Guinea pigs were estrogenized with 0.2 mg/kg diethylstilbestrol intraperitoneally 24 hours prior to *in vitro* drug administration. The uterine muscle of the animal was isolated and contractile responses were obtained using different graded volumes with CASBEEs (1 x 10^{-2} g/ml), oxytocin (1 x 10^{-4} g/ml), ergometrine (1 x 10^{-4} g/ml), and Acetylcholine (1 x 10^{-4} g/ml). The CASBEEs, caused contraction of the isolated estrogenized uterine muscles of the guinea pig in a dose-dependent manner. Maximum contraction (100%) for CASBEEs is at 0.6ml with a final bath concentration of 1.2 x 10^{-4} g/ml. comparatively, maximum contractile responses for the standard drugs was oxytocin 0.8 (1.6 x 10^{-6} g/ml) and ergometrine 0.8ml (1.6 x 10^{-6} g/ml) and Acetylcholine 0.4ml (8 x 10^{-7} g/ml). Aqueous crude extract of the stem-bark of *Erythrophleum suaveolens* exerted a dose-dependent and sustained uterotonic effect on estrogenized isolated uterine muscle of nulliparous Guinea pigs, thus authenticating the folkloric claim for its use to induce labour. Further studies on its mechanism of action and its use for fertility control should be explored.

Keywords: Contraction; Abortifacient; Uterine muscle; Medicinal plant; *Erythrophleum suaveolens*

1 Introduction

The use of herbal medicine has been on the increase in many developing countries and several medicinal plants have been screened for their uterotonic activity using *in vitro* methods with positive results [1]-[2]. Indigenous varieties of plants from various families are used by pregnant mothers in rural areas and low-income populations of sub-Saharan

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Africa for their uterotonic potential [3]. Uterine stimulants are those medicines given to cause contraction of uterus, or to increase the frequency and intensity of the uterine contractions [4].

The World Health Organization (WHO) estimates that traditional and complementary medicine, accounts for 80% of health-care in the world [5]. In the developing countries where modern contraceptive options are limited, >80% of the population continues to use medicinal plants as extract, powder or decoction to prevent unintended pregnancy or in family planning [6]. Lamina *et al*, (2015) also reported the prevalence of unintended pregnancy as 35.9% while that of induced abortion was 33.5% also with the country's economic condition often cited as reasons women (15-48years) make use of abortion services for among women in South-Western Nigeria [7].

Erythrophleum (*Fabaceae- Caesalpinioideae*) is a pantropical woody genus and it has been found to be around 15 species found in Africa, Australia and North-East Asia [8]. *Erythrophleum suaveolens* is a poisonous plant that belongs to the Fabaceae family with distinct foliage. Various reports on medicinal potentials of stem-bark extracts of *E. suaveolens* via scientific screenings, including its anti-oxidant, skeletal muscle relaxation, antibacterial, gastrointestinal motility, anti-fungal, wound healing, anesthetic properties etc have been documented [9 - 12]. Another report by Idyu *et al*., 2014 [13] gave the safety margin of *E. suaveolens* as 223.8±0.05mg/kg bw. From LD_{50} determination. Phytochemical screening of the stem-bark of *E. suaovelens* shows the presence of Tannins, Steroids, Phenols, Terpenoids, Carbohydrates, Saponins, Flavonoids and Phlobatannins (14, 15). Verbal interaction with some natives of the North-Central Nigeria claim that the stem-bark of *E. suaovelens* is often used to induce pregnancy. It is on this premise and the fact that many low incomes earning pregnant women that reported to Hospitals for delivery must have taken one form of herbal preparation or the other that this study aimed at investigating the effect of cold aqueous extract of *Erythrophleum suaveolens* on estrogenized uterus using an animal model.

2 Material and methods

2.1 Collection of plant material, identification and preparation

Fresh plant materials with stem and leaves intact were collected from the Federal College of Forestry Jos, Plateau State, Nigeria. The plant material was identified and authenticated in the same institution. Stem-bark of *E. suaveolens* were harvested, gently rinsed with clean water and dried under shade in a well-ventilated environment. The plant material was ground into powder using mortar and pestle, sieved and stored in dark bottle container until required for use.

Figure 1 *E. suaveolens* Plant [16]

Figure 2 *E. suaveolens* stem bark [14]

2.2 Extraction of Stem-bark of *E. suaveolens*

Powder sample of *E. suaveolens* (100g) was weighed into a glass jar containing 700ml of cold water and stirred thus extracted by maceration for 72 hours. The extract was filtered using Whitman No.1 filter paper. The filtered material was subsequently evaporated to dryness using a rotary evaporator and percentage yield calculated using the formula below. The cold crude aqueous extract was put into dark bottles and stored in a refrigerator at 4°C. These were done according to the method of Idyu *et al.,* 2015 [17] and Builders *et al.,* 2016 [18].

$$\text{Percentage yield} = \frac{\text{Mass of pure powder}}{\text{Mass of impure}} \text{X } 100$$

Percentage yield of the crude extract of *E. suaveolens* (CASBE*Es*) was 14.6%.

2.3 Experimental Animals

Healthy nulliparous Guinea pigs (*Cavia porcellus*) weighing 170–350g were obtained from the Animal House Unit of the Department of Pharmacology and Therapeutics, College of Medicine and Allied Health Sciences, Bingham University, Jos, Nigeria. The animals were housed in plastic cages in the Pharmacology Laboratory of the same Institution, allowed free access to pelleted animal feed and clean water *ad libitum* for 7 days for acclimatization according to the method of Ogundeko *et al.,* 2022 [19].

2.4 Standard Drugs and Physiological Salt solution Used

The following drugs were used for this study:

2.4.1 Estrogen agonist (steroid)

Diethylstilbestrol injection (Kunj Pharma pvt, Ltd., India) was used to estrogenize the nongravid Wistar rats.

2.4.2 Uterotonic drugs

Oxytocin injection (Rotex Medica) and Ergometrine (Hameln. UK.) were used as a standard uterotonic drug.

2.4.3 Cholinomimetic drug

Acetylcholine (Sigma Aldrich Germany).

2.4.4 Physiological salts solution

Freshly prepared De Jalon solution of the following composition: NaCl (9.00g), KCl (0.42g),

$CaCl_2$ (0.06g), $NaHCO_3$ (0.50g) and glucose (0.50g).

2.5 Preparation of Animals and Isolated Organ Preparation

The Guinea pigs were estrogenized with 0.2 mg/kg diethylstilbestrol (DES) intraperitoneally (i.p). The drug was reconstituted with ethanol/water (1:1) solution prior to drug administration. Twenty-four hours later, the animals were sacrificed under chloroform anesthesia, had the uterine horns isolated devoid of excess fat and connective tissues, and cut into longitudinal strips. Then the uterine muscle strips were suspended with one end attached to a tissue holder in a 50 ml capacity tissue bath containing De Jalon's physiological solution. The other end of the strips was connected to an isotonic transducer and in turn connected to a 3 channel microdynanometer Power Lab (BD Instruments – ISO 9001:2000). The entire organ bath was maintained at 37°C and aerated with a mixture of 95% oxygen (O_2) in 5% carbon dioxide (CO_2). Having allowed the preparation to equilibrate for 30 minutes, tissue activity was monitored and observed via the microdynanometer recording paper before and after an intervention. These were also done according to the method described by Ogundeko *et al.,* 2022 [19].

Contractile responses using the following volumes 0.1, 0.2, 0.4, 0.6, 0.8, 1.0 ml were obtained with the CACE*Es* (1 x 10^{-2} g/ml), oxytocin (1 x 10^{-4} g/ml), ergometrine (1 x 10^{-4} g/ml), and Acetylcholine (1 x 10^{-4} g/ml).

3 Result and Discussion

Molecular details of many medicinal plants such as *E. suaveolens* with uterotonic properties are yet to be studied, none the less, those that have been pharmacologically screened and with such activities suggest evident physiological pathways in the human body.

The administration of CASBE*Es*, caused contraction of the isolated estrogenized uterine muscles of the guinea pig in a dose-dependent manner - *Figure 3*. Maximum contraction (100%) for CASBE*Es* was at 0.6ml with a final bath concentration (FBC) of 1.2 x 10^{-4} g/ml. In comparison with the standard uterotonics used, maximum contractile response was oxytocin 0.8ml (1.6 x 10^{-6} g/ml) and ergometrine 0.8ml (1.6 x 10^{-6} g/ml) and Acetylcholine 0.4ml (8 x 10^{-7} g/ml) – Figures 2, 3, 4 and 5 – *Figure 3*.

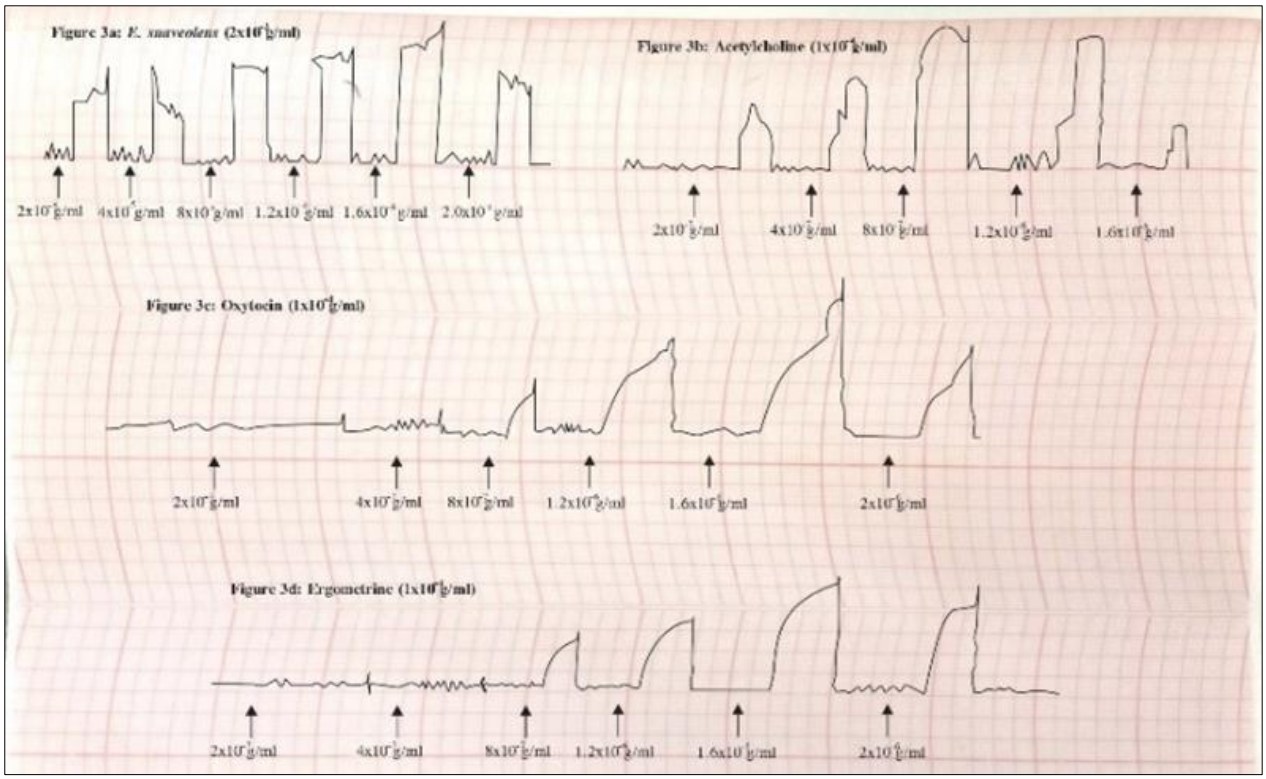

Figure 3 Contractile Response of Estrogenized Isolated Guinea-Pig Uterine Muscle In The Presence Of CASBEEs, Acetycholine Oxytocin and Ergometrine

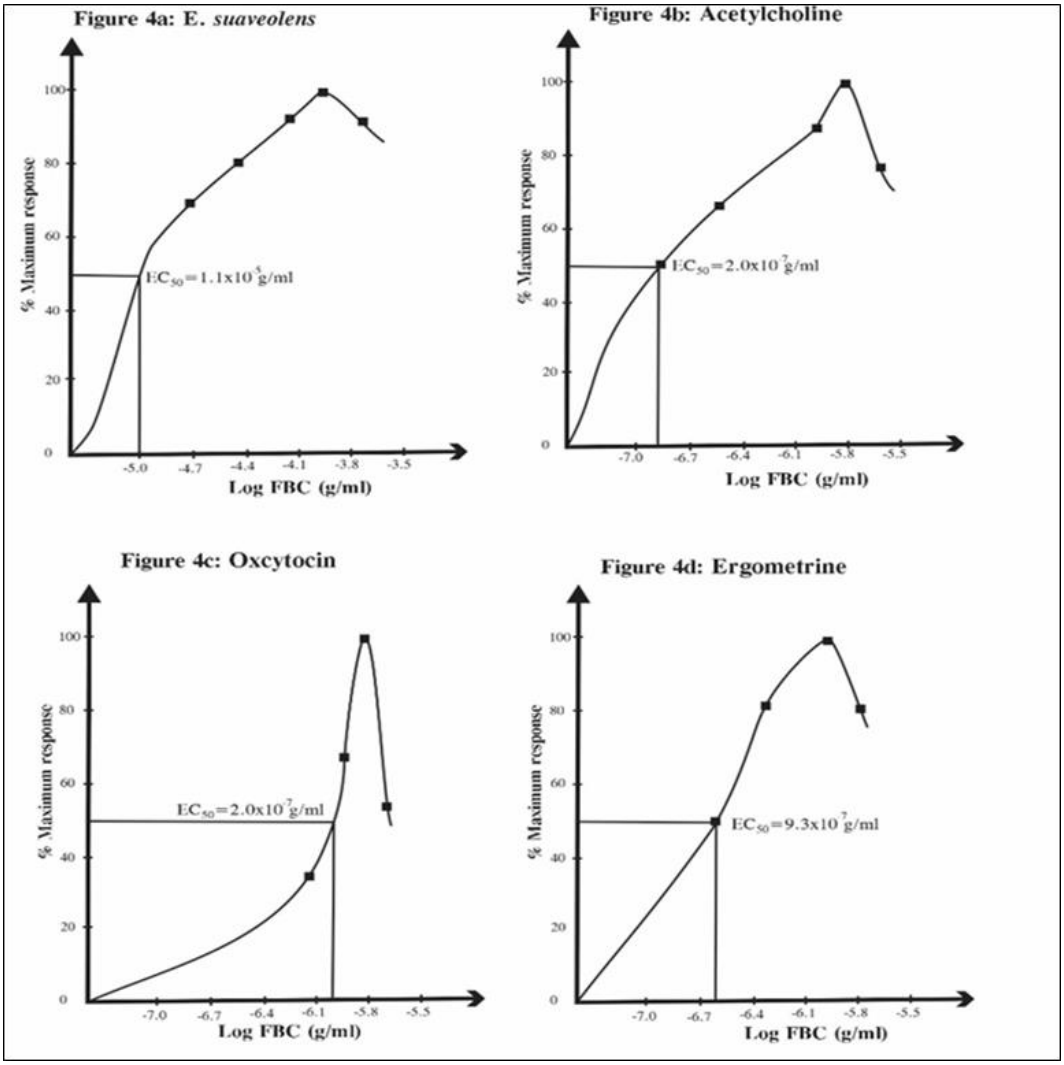

Figure 4 Graphs of Percentage Maximum Response (%) Against Log Final Bath Concent of *E. Suaveolens* Acetylcholine Oxcytocin and Ergometrine

The present study shows that the contractile responses of CASBCE*Es* resembles that of oxytocin with sustained contractions compared with oxytocin and ergometrine.

The graded concentration-response curve gave sigmoidal with EC_{50} for CASBE*Es* (1.1×10^{-5} g/ml), Oxytocin (2.0×10^{-7} g/ml), Ergometrine (9.3×10^{-7} g/ml), and Acetylcholine (2.0×10^{-7} g/ml) – *Figure 4.*

The dose-dependent uterine contraction by CASBE*Es* by way of increase in amplitude and frequency of contraction in the present study is in consonance with the report by Watcho *et al.,* 2011 [7] and Goma et al., 2017 that reported a higher uterotonic potency in cold (aq) than the hot water extract of *Steganotaenia araliacea* Hochst [20]. The use of herbal medicine to alleviate problems associated with gynaecological conditions of menstruation and menopause, to support health during pregnancy and to facilitate childbirth is common amongst many traditional cultures [21].

4 Conclusion

Aqueous crude extract of the stem-bark of *Erythrophleum suaveolens* exerted a dose-dependent and sustained uterotonic effect on estrogenized isolated uterine muscle of nulliparous Guinea pigs, thus authenticating the folkloric claim for its use to induce labour. Further studies on its mechanism of action and its use for fertility control should be explored.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors hereby declare no conflicts of interest with respect to this study.

Statement of ethical approval

Ogundeko T.O, Okoye NP among the authors and Kamoh L are licensed to handle laboratory animals thus, standard protocols involving the use of laboratory animals were strictly adhered to.

References

[1] Rouhi-Boroujeni H, Heidarian E, Rouhi-Boroujeni H, Khoddami M, Gharipour M, Rafieian Kopaei M. Use of lipid-lowering medicinal herbs during pregnancy: A systemic review on safety and dosage. ARYA Atheroscler. 2017: 13(3): 135- 155.

[2] Bwalya P. Factors Influencing Use of Traditional Medicine to Precipitate Labour by Antenatal Mothers in Mpika District. Thesis. University of Zambia, Lusaka, Zambia 2010. cited [2022 Aug 10], Available from http://dspace.unza.zm/handle/123456789/1940

[3] Tripathi V, Stanton C, Anderson FWJ. Traditional preparations used as uterotonics in Sub-Saharan Africa and their pharmacologic effects. *International Journal of Gynecology & Obstetrics*. 2012; 120(1): 16–22.

[4] Roqaiya M, Begum W, Majeedi SF, Saiyed A. A review on herbs with uterotonic property. The J of Phytopharmacol. 2015; 4(3): 190-196.

[5] World Health Organization. WHO traditional medicine strategy: 2014-2023. World Health Organization 2013. cited [2022 Aug 15], Available from https://apps.who.int/iris/handle/10665/92455

[6] Watcho P, Ngadjui E, Nkeng-Efouet PE, Nguelefack TB, Kamanyi A. Evaluation of *In Vitro* Uterotonic Activities of Fruit Extracts of *Ficus asperifolia* in Rats, *Evidence-Based Compl and Alt Med*. 2011; 1: 783413.

[7] Lamina MA. Prevalence and Determinants of Unintended Pregnancy among Women in South-Western Nigeria. Ghana Med J. Sep 2015; 49(3): 187-94.

[8] Duminil J, Brown RP, Ewedje EEB, Mardulyn P, Doucet JL, Hardy OJ. Large-Scale Pattern of Genetic Differentiation within African Rainforest Trees: Insights on the Roles of Ecological Gradients and Past Climate Changes on the Evolution of *Erythrophleum spp.* (Fabaceae). *BMC Evol. Biol. 2013; 13*: 195.

[9] Idyu II, Olaoye T O, Sokomba E N, Idyu VC, Ramyil MS, Ogbole EA, Builders MI, Ogundeko TO. The pharmacological evaluation of cold water stem-bark extract of *Erythrophleum suaveolens* on gastrointestinal muscle of guinea-pig (*Cavia porcellus*) Ileum. Int J Sci Res. 2014; 3(5): 1-6.

[10] Akinpelu BA, Dare CA, Adebesin FI, Iwalewa EO,Oyedapo OO. Effect of stem – bark of Erythrophleum suaveolens (Guill. & Perri.) saponin on fresh water snail (Lanistes lybicus) tissues. Afric. J.Environmental Sci. & Tech. 2012; 6(11): 446-451.

[11] Ogundeko TO, Idyu II, Ojo MT, Idyu VC, Ogbole EA, Builders MI, Ramyil MS, Toma BO, Asalu AF. Investigation of Relaxative Effect of Stem-Bark Extract of Erythrophleum Suaveolens on Rat Phrenic Nerve-Diaphragm Muscle. *JAMPS.* 2015; *3*: 24-30.

[12] Akanji, OL, Sonibare MA. Evaluation of wound healing activity of Erythrophleum suaveolens (Guill.and Perr.) brenan and Moringa oleifera (Lam) on infected albino Rats. Europ J of Medl Plants. 2015; 7(2): 6776.

[13] Idyu II, Kela SL, Idyu VC, Akinyede A, Builders MI, Ogbole EA, Ramyil MS, Ogundeko TO. Acute Toxicity Studies of *Erythrophleum suaveolens* in Albino Mice (Mus musculus). IJSR. 2014; 3: 366-371.

[14] Yusuf HO, Olu J, Akhigbe CI. (2022). Assessment of phytochemical constituents of *Erythrophleum suaveolens* (Brenan) stem bark extracts and its biological control of *Aspergillus flavus* isolated from *Zea mays.* J of Biotech. 2022; 1(1): 37-45.

[15] Hassan SW, Ladan MJ, Dogondaji, RA, Umar R.A, Bilbis LS Hassan LG, Ebbo AA, Matazu IK. Phytochemical and toxicological studies of aqueous leaves extract of *Erythrophleum africanum*. *Pakistan J of Biocal Sci*. 2007; *10*(21): 3815-3821.

[16] African plants: A photo guide-E. Suaveleons plant. cited [2022 Aug 12], Available from https:// africanplans.senckenberg.de/root/index.phpp?page_id=78&id=676

[17] Idyu II, Deshi EF, Idyu VC, Ogundeko TO. The Anti-diarrhoeal Effect of Ethanolic-Bark Extract of *Sterculia setigera* in Mice. *Asian J. Sci.Tech.* 2015; 6(5): 1397-1400.

[18] Builders MI, Builders PF, Ogundeko TG. Anti-ulcer activity of the stem bark of African locust bean tree in rats. *Int. J. Phytotherapy Research*. 2016; 6(4): 11-19.

[19] Ogundeko TO, Osagie IA, Bitrus J, Okoye N P, Ogbole EA, Edugbe AE, Abobarin OI, Moritiwon O, Ramyil MSC, Bassi AP. The uterotonic screening of calyx extracts of *Hibiscus sabdariffa* on estrogenized isolated Uterus of Wister strain albino rats. Int J Frontiers in Bio and Pharmacy Res. 2022; 3(1): 037–045.

[20] Goma FM, Ezeala C, Nyirenda J, Chuba D, Prashar L, Simfukwe N, Lengwe C. Extraction and Demonstration of Uterotonic Activity from the Root of *Steganotaenia araliacea* Hochst. Medical Journal of Zambia. 2017; 44(3): 125-132.

[21] Gruber CW, O'Brien M. Uterotonic plants and their bioactive constituents. Planta Med. Feb 2011; 77(3): 207-20.