Uterine Contractile Activity and Abortifacient Effect of the Aqueous Extract of *Euphorbia heterophylla* L. Leaves in Albino Rats

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ABSTRACT

Background and Purpose: *Euphorbia heterophylla* Linn. (Euphorbiaceae) is a promising medicinal plant used as an oxytocic in folkloric medicine. The study investigated the uterotonic and abortifacient effects of the aqueous leaf extract of *Euphorbia heterophylla* (AEEH).

Methods: GC-MS analysis of AEEH was done. *In vitro* experiment on the contractile activity of AEEH was conducted on the uterus of nulliparous female Sprague Dawley rats using an isolated organ bath. The abortifacient activity of the extract was determined in pregnant rats orally administered 50, 100, 200, and 400 mg/kg AEEH for five consecutive days.

Results: GC-MS analysis of AEEH revealed high amounts of 1-butanol 3-methyl acetate, benzofuran, 2,3-dihydro-, 2-methoxy-4-vinyl phenol, and sucrose. Some of the classes of compounds identified in the extract such as phenols, terpenoids, fatty acids, aldehydes, steroid, and glucoside have been associated with uterotonic and abortifacient properties. The extract (0.125-4 mg/mL) was able to enhance the contractile activity of uterine smooth muscles ($EC_{50} = 3.39 \times 10^{-1}$ mg/mL) similar to oxytocin ($EC_{50} = 3.99 \times 10^{-4}$ mg/mL) and acetylcholine ($EC_{50} = 2.68 \times 10^{-4}$ mg/mL). Pre-treatment with salbutamol, indomethacin, and stimulation in a calcium-deprived medium significantly ($P < 0.0001$) reduced uterine contractions induced by cumulative concentrations of the aqueous extract. However, there was no significant change in uterine contraction in tissues pre-treated with atropine. The aqueous extract induced abortion in 50%, and 83.33% of the animals at 200, and 400 mg/kg respectively when orally administered to pregnant rats for five days.

Conclusions: This study demonstrates the uterotonic and abortifacient effects of AEEH and supports the ethnomedicinal use of the plant as an oxytocic.

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INTRODUCTION

The use of herbs as uterine stimulants is more common in developing countries because of the ease of accessibility, affordability, and efficacy of these plants (Illamola *et al.*, 2020). Notwithstanding the use of uterotonic plants in most developing countries, a lot of women suffer childbirth-related complications. Annually, 99% of about 300,000 maternal deaths occurring worldwide are in developing countries (Tadele and Abebaw, 2017). The inappropriate use of uterotonic plants due to inadequate scientific knowledge on their safety, efficacy, and mode of action contributes to the increase in maternal mortality (Zamawe *et al.*, 2018). Uterotonic plants are effective for inducing labour, augmenting slowly progressing labour, preventing postpartum haemorrhage, and removal of retained placenta after childbirth. They are also commonly used as abortifacients because abortion remains illegal in most developing countries, and most women readily find alternative means of committing abortion outside the health care system. The varieties of bioactive constituents present in uterotonic plants are capable of acting on various physiological targets and eliciting contraction in the myometrial cells (Gruber and O'Brien, 2011).

Euphorbia heterophylla Linn that belongs to the family Euphorbiaceae is a common weed found in tropical, arid, and semi-arid regions of the world (Hutchinson and Dalziel, 1958). In South-western Nigeria, the plant is referred to as "Adimeru". It is used in the treatment of typhoid, constipation, skin lesions, malaria, and migraine (Kone and Onifade, 2020). Scientific investigations have confirmed its laxative, antioxidant, anti-inflammatory, antimicrobial, and cytotoxic properties (Oyedum, 2017; Wellington *et al.*, 2020). The leaves are also used as abortifacients due to the uterine stimulating properties (Unekwe *et al.*, 2006). However, the abortifacient effect has not been sufficiently explored. Thus, this study aimed at investigating the abortifacient effect, uterine contractile activity, and determining the chemical constituents present in the aqueous extract of *E. heterophylla* leaves.

MATERIALS AND METHODS

Plant Material Collection and Identification

In March 2021, *E. heterophylla* leaves were obtained from a farm in the Federal University of Agriculture, Abeokuta (collection site: longitude 7.2437°N and latitude 3.3433°E). The leaves were harvested in the rainy season, with average humidity and temperature of 85% and 30°C respectively. A specimen of the fresh plant was identified by Mr. Oyebanji, a taxonomist and forestry expert at the herbarium of the Department of Botany, University of Lagos, Nigeria. It was allocated a voucher specimen number 6958. The plant was also checked with The Plant

List, a list of various plant species at <http://www.theplantlist.org>.

Plant Material Extraction

The collected leaves of *E. heterophylla* were rinsed with deionized water, gently blotted with a paper towel, and dried at a room temperature of 27°C. The dried leaves were ground into powder using a dry mill blender (Binatone, China). The powdered sample (100 g) of the leaf was soaked in water (2 L) at 100°C, allowed to stand at room temperature (27°C) for 1 h, and filtered. The filtrate was concentrated with a water bath (Scientific Laboratory Supplies, England) at 50°C. The extract was weighed (percentage yield = 26.95% w/w) and kept in an airtight container at 4°C till further use.

GC-MS Analysis of the Aqueous Leaf Extract of *E. heterophylla* (AEEH)

Chemical compounds in the aqueous leaf extract were analysed using a GC-MS (GC-MS-QP2010SE Shimadzu, Japan) adopting the method used by Sulaimon *et al.* (2020). The sample was dissolved in ethanol and 0.2 µL was injected into the system. The injection temperature was placed at 250°C and helium was used as the carrier gas. The temperature of ion source was positioned at 230°C. The experiments were carried out on a positive ion mode with a total running time of 29 min. The detection of compounds was based on the NIST (National Institute of Standards and Technology) library, 2011.

Experimental Animals

Healthy mature Sprague-Dawley female and male rats aged between 2 and 3 months, and weighing between 150 and 170 g were housed in the animal house of the College of Medicine, University of Lagos. They were kept in plastic cages at a managed room temperature of between 27-30°C with a light-dark cycle of 12 h. The animals were fed with a standard diet and given clean water *ad libitum* and were acclimatized for seven days. The study conformed to the principle of laboratory animal care guidelines and procedures (NIH publication revised, No.85-23, 1985). Animal use and care complied with international laboratory animal use and care guidelines. Ethical approval for the use of the animals was obtained from the College of Medicine, University of Lagos Health Research Ethics Committee (CMUL HREC) and given a protocol number CMUL/ACUREC/12/20/797 on January 18, 2021.

Drugs and Chemicals

Sodium bicarbonate (99.5% purity), sodium chloride (99% purity), glucose (99.5% purity), potassium chloride (99% purity), calcium chloride (97% purity), and EDTA (98.5%

purity) for the physiological salt solution were obtained from Sigma Aldrich (Germany). The drugs used in the study include estradiol valerate (2 mg, Bayer Plc, England), oxytocin (10 I.U, Shuguang Pharm Co Ltd, China), indomethacin (25 mg Maxheal Pharmaceuticals Ltd, India), salbutamol (2 mg, Greater Pharma, Thailand), misoprostol (200 µg, Pfizer, United States), atropine sulfate (600 µg/mL Hameln Pharma Ltd, England) and acetylcholine chloride (98% purity, Reagecon Diagnostics Ltd, Ireland).

Uterine Contractility Studies

Uterine tissue preparation

The uteri of healthy female rats were estrogenized by pretreatment with subcutaneous injection of 0.1 mg/kg estradiol valerate 24 h before the experiment, according to the method described by Nworgu *et al.* (2015). The rats were then sacrificed and the uterine horns harvested. The uterine horns were deposited in an aerated and warmed De Jalon physiological salt solution composed of (g/L): NaCl, 9.0; Glucose, 0.5; NaHCO₃, 0.5; MgCl₂, 0.006; KCl, 0.42; CaCl₂.2H₂O, 0.08. Each uterine horn was suspended in a 50 mL organ bath containing the physiological salt at a controlled temperature of 37°C and aerated with 5% CO₂ in O₂. The methods used were similar to those described by Bafor *et al.* (2018). The uterine strips were attached to an isometric force-displacement transducer coupled to a PowerLab and a LabChart 7 software (all from AD Instruments, New Zealand) that measured the uterine contractions. The transducer was calibrated to establish a relationship connecting the force applied to the transducer and the gauge deflection with a corresponding weight of 1 g. Each preparation was let to equilibrate for 45 min prior administration of the extract or drugs.

Drug challenges

After 45 min of equilibration, the effects of cumulative concentration of AEEH (0.125-4 mg/mL), oxytocin (0.1-1.6 IU) and acetylcholine (1×10⁻⁴ - 1.6 ×10⁻³ mg/mL) on the spontaneous uterine contractions were measured. The amplitude of contraction was recorded till a plateau was reached. Salbutamol (1 mg/mL), indomethacin (1 mg/mL), and atropine (0.12 µg/mL) were used in a dose-dependent approach to antagonize the uterine contractions induced by AEEH (0.125-4 mg/mL), 5 min before the addition of the extract. The effect of AEEH (0.125-4 mg/mL) on uterine contractions was also observed in calcium-free De Jalon solution, by the removal of calcium chloride and addition of ethylenediaminetetraacetic acid, EDTA (0.1 mM). The tissues were washed thrice and let to rest for 30 min in between extract or drug administration. Oxytocin conversion from international units (IU) to µg/mL was done following the World Health Organization (WHO)

recommendation where 10 IU = 16.6 µg/mL (WHO, 2016). The experiment was replicated 6 times for the drug challenge.

Determination of the Abortifacient Activity

The abortifacient activity of AEEH was determined in pregnant rats by adopting the method used by Namulindwa *et al.* (2015). Female virgin Sprague-Dawley rats were mated with male Sprague-Dawley rats by placing them in cages at a ratio of 2:1. They were observed every morning for the presence of a dislodged sperm plug which was taken as day 1 of pregnancy. The pregnant animals were randomly assigned to six groups consisting of six rats each. Group 1 received 5 mL/kg/day normal saline orally. Groups 2, 3, 4, and 5 were orally administered 50, 100, 200, and 400 mg/kg/day respectively of AEEH. Group 6 received misoprostol (100 µg/kg/day) orally. The treatment commenced on the fifth day of pregnancy and continued for five consecutive days. The animals were observed for signs of abortion (bleeding or fetal expulsion) for the next five days. On the tenth day of pregnancy, animals were sacrificed and opened up for observation of the number of fetuses. Absence of fetuses confirmed that abortion had occurred. The number of fetuses and the percentage of rats that aborted were calculated using the formula:

$$\% \text{ of rats that aborted} = \frac{\text{Number of rats that aborted in group}}{\text{Total number of rats in the group}} \times 100$$

Statistical Analysis

Statistical analysis was done using GraphPad Prism 6 (Version 6.01, GraphPad software inc, Ca, USA). Results are expressed as mean ± standard error of mean (S.E.M) or mean ± standard deviation (SD) of 6 replicates and were subjected to t-test, and one-way analysis of variance followed by Dunnett's multiple comparison test. Values were regarded as statistically significant at $P < 0.05$.

Mean log concentration curve was plotted by inserting data into a variable slope logistic equation applying the equation value:

$$Y = \frac{\text{Bottom} + (\text{Top} - \text{Bottom})}{(1 + 10^{((\text{Log } EC_{50} - X) \times \text{HillSlope}))}}$$

Where Y= response that starts at the bottom and goes to the top in sigmoid shape, X= logarithm of the concentration, EC₅₀ (half-maximal effective concentration) = concentration that gives half of the maximal response.

RESULTS

GC-MS Analysis of the Aqueous Leaf Extract of *E. heterophylla*

The identified compounds in the plant extract, with the retention time, peak area percentage, molecular weight, and class are presented in Table 1. The total ion

chromatography of the plant extract is presented in Figure 1. The most abundant compounds in the plant extract were 1-butanol 3 methyl acetate (number 20) followed by sucrose (number 26), 2-Methoxy-4-vinyl phenol (number 25), and benzofuran,2,3-dihydro- (number 22).

Table 1: Bioactive components identified through GC-MS analysis of aqueous leaf extract of *E. heterophylla*

Peak	Retention time	Area (%)	Molecular Weight	Compound Name	Class
1	4.974	1.83	98	Furfuranol	Alcohol
2	5.225	0.07	115	Propanamide, N- Acetyl	Amide
3	5.287	1.32	86	Butyrolactone	Lactone
4	5.330	0.14	108	Pyrazine, 2,5-dimethyl-	Diazine
5	5.365	0.32	100	Ethanol 2,-(2-propylnyloxy)-	Alcohol
6	5.469	0.91	134	3,4-Epoxytetrahydrothiophene-1,1-dioxide	Thiolane
7	5.543	0.85	128	Cyclohexanone	Cyclic ketone
8	5.787	0.23	102	Octanal	Aldehyde
9	6.024	0.75	102	2-hydroxy-gamma-butyrolactone	Lactone
10	6.099	0.34	102	2-hydroxy-gamma-butyrolactone	Lactone
11	6.183	0.36	140	Cyclopentane-1,2-diol	Cyclic alkane
12	6.315	0.40	113	9-Oxabicyclo [6.1.0] nonan-4-one	Cyclic ketone
13	6.462	0.18	112	Ethyl cyanoacetate	Carboxylic acid ester
14	6.553	0.26	116	1,2 cyclopentanedione, 3-methyl-	Cyclic ketone
15	6.643	0.39	128	2-hexanone,6-hydroxy-	Ketone
16	6.680	0.29	85	5H-1,4-Dioxepin, 2,3-dihydro-2,5-dimethyl-	-
17	6.893	0.84	128	2,5-Dimethyl-4-hydroxy-3 (2H)-furanone	Lactone
18	7.046	2.23	86	Pentanal	Aldehyde
19	7.127	0.24	128	6,7- Dioxabicyclo[3.2.2]nonane	Cyclic alkane
20	7.820	55.20	130	1-Butanol, 3-methyl, acetate	Isoamyl acetate
21	8.336	0.64	110	Hydroquinone	Phenol
22	8.474	4.45	120	Benzofuran,2,3-dihydro-	Essential oil
23	8.923	0.08	141	3,6-Dimethyl-3,6-digydro-pyran-2-one oxime	-
24	9.201	0.17	254	9-Tetradecen-1-ol, acetate,(E)-	Alcohol
25	9.470	7.30	150	2-Methoxy-4-vinylphenol	Phenol
26	11.412	16.22	342	Sucrose	Disaccharide
27	12.154	0.21	178	3-Tridecen 1-yne, (E)-	Alkyne
28	12.267	0.20	168	7-Oxabicyclo [4.1.0] heptane. 1-methyl-4-(2-methyloxiranyl)-	Alkane
29	12.475	0.12	177	Phenol, 2-amino-4- (1H- 1,2,3,4-tetrazol-1-yl)-	Phenol
30	12.711	0.18	280	3-Methyl-4-(phenylthio)-2 -2,5-dihyprop-2-enyl-2,5-dihydrothiophene 1,1-dioxide	Thiophene
31	12.922	0.13	164	3-Dodecene-1-yne, (E)-	Alkyne
32	13.053	0.08	328	Spiro [androst-5-ene-17, 1'cyclobutan]-2'-one, 3	Steroid
33	13.124	0.06	280	1-Gala-1-ido-octose	Monosaccharide
34	13.450	0.07	168	7-Oxabicyclo [4.1.0] heptane. 1-methyl-4-(2-methyloxiranyl)-	Cyclic alkane
35	13.781	0.10	209	1,4-Methano-1H-cyclohepta[d]pyridazine,4 4a	Diazine
36	13.878	0.09	209	Bicyclo [2.2.1] heptan-2-one,4,7,7-trimethyl semicarbazone	Ketone
37	14.053	0.04	180	1,2,4-cyclopentanetrione,3-(2-pentenyl)-	Cyclic ketone
38	12.281	0.33	170	4-Isobenzofuranol, octahydro-3a,7a-dimethyl-,	Alcohol
39	14.432	0.25	164	1- (3,6- Dimethyl-2-pyrazinyl) propanone	Ketone
40	14.628	0.05	224	2-Pentadecyn-1-ol	Alcohol
41	14.737	0.15	164	2 (1H)- Naphtalenone, 4a,5,6,7,8,8a-hexahydro-4a-methyl-	Ketone
42	15.221	0.12	190	Syn-tricyclo[5.1.0.0 (2,4)]oct-5-ene, 3,3,5,6,8,8-hexamthyl-	Cyclic Alkene

Table 1 (Continued): Bioactive components identified through GC-MS analysis of aqueous leaf extract of *E. heterophylla*

43	15.475	0.03	300	Androsta-1,4-dien-3-one, 17-hydroxy-17- methyl-, (γ -17.alpha.)-	Terpenoid
44	15.545	0.16	350	Andrographolide	Terpenoid
45	15.814	0.09	224	2- Pentadecyn-1-ol	Alcohol
46	15.953	0.05	114	Heptanal	Aldehyde
47	16.282	0.21	145	.gamma.-Guanidinobutyric acid	Fatty acid
48	16.446	0.31	312	Eicosanoic acid	Fatty acid
49	16.804	0.39	186	Undecanoic acid	Fatty acid
50	17.816	0.05	156	Decanal	Aldehyde
51	18.120	0.26	284	Octadecanoic acid	Fatty acid
52	18.483	0.21	270	Benzyl. beta-d-glucoside	Glucoside
53	20.414	0.04	354	3-Isopropoxy-1,1,1,5,5,5-hexamethyl-3-(trimethylsiloxy) trisiloxane	-

Figure 1: Total ion chromatograph of the aqueous extract of *E. heterophylla* leaves

Effect of Aqueous Leaf Extract of *E. heterophylla* and Drugs on Uterine Contractility

The results showed that AEEH induced uterine contractions at a higher EC_{50} value (3.39×10^{-1} mg/mL) compared to the EC_{50} values of oxytocin (3.99×10^{-4} mg/mL) and acetylcholine (2.68×10^{-4} mg/mL).

Figure 2 shows the representative tracings and Figure 3 shows the concentration-response curves. The uterotonic activity of the AEEH was significantly ($P < 0.0001$) reduced in the presence of salbutamol (SAL), indomethacin (IND), and a calcium-free media ($0Ca^{2+}$) (Figure 4). Atropine (ATP) did not cause significant changes in the uterine contractile activity of the extract.

Figure 2: Representative graph tracings showing the uterine contractile activity by oxytocin (a), acetylcholine (b) and aqueous leaf extract of *E. heterophylla* (c). The tissues were washed in between cumulative doses of extract and drugs.

Figure 3: Concentration-response curves representing effects of increasing concentrations of aqueous leaf extract of *E. heterophylla* (0.125 - 4 mg/mL), oxytocin (0.1-1.6 IU), and acetylcholine (1×10^{-4} - 1.6×10^{-3} mg/mL) on the amplitude of uterine contractions. The EC_{50} value for aqueous leaf extract of *E. heterophylla*, oxytocin, and acetylcholine were 3.39×10^{-1} mg/mL, 3.99×10^{-4} mg/mL and 2.68×10^{-4} mg/mL respectively. AEEH: aqueous leaf extract of *E. heterophylla*, OXT: oxytocin, ACH: acetylcholine. EC_{50} : half maximal effective concentration.

Figure 4: The effect of AEEH on uterine contractions in tissues pre-treated with indomethacin (a) salbutamol (b) atropine (c) and in a calcium-free media (d). * $P < 0.0001$ (t-test), INDO: indomethacin, SAL: salbutamol, ATR: atropine AEEH: aqueous leaf extract of *E. heterophylla*, (0Ca²⁺): aqueous extract of *E. heterophylla* leaves in a calcium-free physiological solution.

Abortifacient Effect of the Aqueous Leaf Extract of *E. heterophylla*

The result on the abortifacient effect of the extract is presented in Table 2. Abortion was not observed in the control group and extract-treated groups at 50 and 100 mg/kg/day for the five days. However, the plant extract

induced abortion in 3 out of 6 rats (50%) administered 200 mg/kg/day, and 5 out of 6 rats (83.33%) administered 400 mg/kg/day. Misoprostol (100 µg/kg/day) induced abortion in all the animals (100%). The mean number of fetuses was significantly decreased ($P < 0.0001$) in the misoprostol (100 µg/kg) treated group and extract-treated group at 200 and 400 mg/kg.

Table 2: Effect of different doses of the aqueous leaf Extract of *E. heterophylla* in pregnant rats

Groups	Number of Pregnant Rats	Number of Fetus in Individual Rats	Mean Number of Fetus	Number of Rats that Suffered from Abortion	Percentage of Rats that Suffered from Abortion
Control	6	8,9,7,10,8,7	8.17±1.17	0	0.00
50 mg/kg/day AEEH	6	7,8,8,10,8,7	8.00±1.10	0	0.00
100 mg/kg/day AEEH	6	7,6,7,7,7,8	7.00±0.63	0	0.00
200 mg/kg/day AEEH	6	5,4,0,0,0,3	2.00±2.28****	3	50.00
400 mg/kg/day AEEH	6	0,3,0,0,0,0	0.50±1.23****	5	83.33
Misoprostol (100µg/kg/day)	6	0,0,0,0,0,0	0.00±0.00****	6	100

Results are represented as mean ± SD (n=6) **** $P < 0.0001$ AEEH: aqueous leaf extract of *E. heterophylla*

DISCUSSION

The present study demonstrates the uterotonic activity of AEEH on the isolated rat uterus. The plant extract increased the force of uterine contractions ($EC_{50} = 3.39 \times 10^{-1}$ mg/mL), thereby exhibiting efficacy. However, oxytocin ($EC_{50} = 3.99 \times 10^{-4}$ mg/mL) and acetylcholine ($EC_{50} = 2.68 \times 10^{-4}$ mg/mL) showed greater potency. The uterotonic activities of these standard agents and the pathways for contraction of the myometrium are well established (Arrowsmith and Wray, 2014; Jana *et al.*, 2019). A variety of other medicinal plants have also shown uterotonic potential similar to the plant extract in this study: the fruits of *Ficus asperifolia* (Watcho *et al.*, 2011), rhizomes of *Nymphaea alba* (Bose *et al.*, 2014), leaves of *Spondias mombin* (Pakoussi *et al.*, 2018), roots of *Azanza garckeana* (Chanda *et al.*, 2020), and leaf extract of *Sida corymbosa* (Bakut *et al.*, 2020). The uterotonic potential of *E. heterophylla* has also been confirmed by Unekwe *et al.* (2006), however the mechanism of action of the plant has been extensively explored in this present study.

Various pathways are involved in the stimulation of uterine contractions by uterotonic plants (Arrowsmith *et al.*, 2010; Bafor and Kuppitnayant, 2020). The adrenergic pathway requires stimulation of α_1 adrenergic receptor or inhibition of β_2 adrenergic receptors in mediating uterine contractions. The cholinergic pathway solely involves muscarinic receptors. Acetylcholine elicits uterine contractile response by acting as an agonist to muscarinic receptors. Prostaglandin pathways use the synthesis of prostaglandins (the mechanism also used by oxytocin receptors) or stimulation of prostaglandin receptors (PGF $_{2\alpha}$ and PGE), in stimulating uterine contractions. The flow of extracellular calcium through the voltage-gated L- type calcium channels, and intracellular calcium from the sarcoplasmic reticulum, also facilitate myometrial contractions.

The action of various drugs and compounds on various receptor sites on myometrial cells has enabled the

elucidation of the mechanism of most uterotonic plants. Salbutamol, a β_2 adrenergic receptor agonist is a smooth muscle relaxant used as a tocolytic to delay premature labour (Zulfiqar and Iftikhar, 2016). Atropine, a non-specific muscarinic receptor antagonist also has a smooth muscle relaxant effect and reduces the uterine contractile effect of acetylcholine (Kodama *et al.*, 2010). Indomethacin, a prostaglandin synthesis inhibitor is often used to reduce the severity of uterine contractions in women suffering from dysmenorrhea (Marjoribanks *et al.*, 2015). The significant reductions in the force of uterine contractions by AEEH in the presence of salbutamol and indomethacin indicates possible antagonism of β_2 adrenergic receptor and inhibition of prostaglandin synthesis as the pathways involved in uterine contraction induced by the plant extract. The absence of significant changes in uterine contractions induced by AEEH in the presence of atropine suggests that cholinergic pathways may not be involved. Calcium plays an essential role in uterine contraction. The decrease in uterine contractions in a totally calcium-deprived medium suggests that the mobilization of extracellular calcium is actively involved in the uterine contractile activity of AEEH. Other uterotonic plants with similar mechanisms of action include *Spondias mombin*, *Helichrysum mechowianum* and *Heimia salicifolia* (Cobos *et al.*, 2019; Bafor and Kuppitnayant, 2020).

The abortifacient activity of AEEH was majorly achieved at the higher doses of the extract. Vaginal bleeding was observed in some animals 24 h after administration of the extract. The absence of fetuses in the uteri of some animals at the end of the study further confirmed abortion. This supports the folkloric use of the plant as an abortifacient. The aqueous method of extraction was used in this study to depict a mode of extraction similar to its traditional use. The basis for selection of doses involved using a starting dose that was 1/40th of the dose (2000 mg/kg) associated with liver toxicity in a previous acute toxicity study (Wellington *et al.*, 2020) and using subsequent doses (100, 200 and 400 mg/kg/day). The doses used in this study also covered the range of doses

(100-300 mg/kg) reported to have biological activities from previous efficacy studies (Falodun *et al.*, 2006; Annapurna and Hatware, 2014; Omoya *et al.*, 2015). This creates more awareness on the side effects of the extract particularly in pregnant women using it for other therapeutic purposes.

The uterotonic and abortifacient potential of AEEH are dependent on the active compounds present in the plant. GC-MS analysis showed the presence of 53 compounds in the extract. Compounds such as 1-butanol 3 methyl acetate, sucrose, 2-methoxy-4-vinyl phenol, and benzofuran, 2,3-dihydro-. abundantly found in the extract, have been reported to have antimicrobial, antioxidant, cytotoxic, analgesic, and anti-inflammatory properties (Ando *et al.*, 2015; Ibibia *et al.*, 2016; Petrova *et al.*, 2018). Some of the compound classes detected in the plant extract, such as phenols, terpenoids, steroid, glucoside, and fatty acids have been reported to have smooth muscle contractile and cytotoxic properties that are connected to their uterine contractile and abortifacient properties (Gruber and O'Brien, 2011; Ijioma *et al.*, 2020; Nie *et al.*, 2021). Phenolic compounds particularly 2-methoxy-4-vinyl phenol identified in the GC-MS analysis of *E. heterophylla* extract have been identified in various abortifacient plants such as *Lawsonia inermis*, *Tephrosia falciformis*, and *Balanites aegyptiaca* (Rajeswari and Rani, 2015; Vandana and Deora, 2020; Murthy *et al.*, 2021). Exposure to phenolic compounds has been associated with spontaneous abortion (Chen *et al.*, 2013). Thus, phenols may majorly contribute to the abortifacient properties of *E. heterophylla*.

CONCLUSION

The aqueous extract of *E. heterophylla* possesses uterotonic and abortifacient effects. The diverse classes of bioactive compounds present in the extract especially phenols, terpenoids, aldehydes, fatty acids, steroid and glucoside are likely responsible for the uterotonic and abortifacient properties of the plant. This study justifies the use of the plant as an abortifacient. Further studies regarding the use of other extraction methods and isolation of bioactive compounds responsible for the uterine contractile properties are recommended.

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AUTHOR CONTRIBUTIONS

OOE conceptualized the study, conducted the experiments, analysed data, and wrote the manuscript. VAN and JUO assisted in the experimental design and reviewed the manuscript. The entire study was supervised by AAA and IOO. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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