



Abortifacient efficacy of aqueous-acetone extracts of *Adenopus breviflorus* Benth seed in female albino rats

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ABSTRACT

The present study evaluated the abortifacient potential of aqueous-acetone extract of *Adenopus breviflorus* Benth Seed on the reproductive health of matured female albino rats by monitoring the indices of dysfunctions in reproductive system. Prior to the conduction of the fertility studies, the oral acute toxicity of the seed extract was evaluated for autonomic, behavioral and neurological changes, within 24 h to determine the LD₅₀. The influence of aqueous-acetone extract of *Adenopus breviflorus* Benth Seed was evaluated for antioxidant, reproductive hormones and histology of uteri tissues primarily to monitor effects in female fertility. Female rats exhibiting thick clump of spermatozoa in their vaginal smear were randomly selected and used for the study to determine the abortifacient activity of the seed extract. Parameters such as number of live and dead fetuses, anogenital distance (AGD) and crown rump length (CRL), and the variation in birth weight of litters and gestation period between control and experimental animals were determined. The phytochemical composition of the seed extract was characterized by Gas Chromatography and Mass Spectrophotometry for the identification of phytochemical of toxic or therapeutic effects. Symptoms similar to clinical toxicity such as salivation, respiratory distress, weight loss and change in appearance of hair were noticed at concentrations above 1600 mg/kg BWT, there was no maternal mortality at any period of the experiment. There were changes in the behavioural, neurological and autonomic profile in groups with doses greater than 1600 mg/kg BWT. The LD₅₀ evaluation showed that the aqueous-acetone extract of the seed was safe at dose of 5000 mg/kg BWT. The GC-MS characterization of the aqueous-acetone seed revealed isomeric derivatives of benzene-mesitylene and pseudocumene 4.28 g/100 g of sample) and 5.85 g/100 g of sample respectively as most predominant phytochemicals in the seed extract which demonstrated maternal toxicity. The effects on the female reproductive hormones of the treated animals revealed that FSH, LH and prolactin were significantly reduced ($p < 0.05$) in all the treated groups by the extract. Progesterone (PH) and estrogen (EH) were also reduced significantly. The study revealed scientific evidence in support of the abortifacient activity of seed extract that was significantly corresponding to the discovered phytochemical compounds.

1. Introduction

The dependence of human on herbs for pharmacological interventions could result into toxic outcomes, these toxic outcomes persisted before the advent of orthodox medicine. Prior to civilization, efforts have been channeled into designing synthetic drugs from bioactive compounds of known safety dose, function and mechanism in medicinal plants without toxic effects.

Some plants had been reported as contraceptive (impairment of ovulation or fertilization), abortifacients (impairment of implantation), anti-progesterone and emmenagogues (impairment of uterine flow) or oxytocics (stimulation of uterine contractions vis-a-vis promotion of labour) [1]. There were sparse information on the pre-civilization use of the herbs in birth control or female fertility. Folkloric herbal alternatives are easily accessible compared to the latent quest for orthodox oral contraceptive agents especially in societies where there are laws and

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regulations preventing the sale, purchase and use of over the counter (OTC) orthodox contraceptive drugs which can be employed for controlling human fertility. Leaves of *Milletia aboensis* [2] and *Indigofera trifoliata* [3] had demonstrated estrogenic potencies by impairing the secretion of these fertility hormones as well as blocking ovulation, they may intercept the synchronized development of the ovum and endometrium. These plants influenced the pituitary action by the auxiliary modulation of gonad hormones via impairment in hormonal output and ovulation as well as interference with the synchronized development of the ovum and the endometrium.

Adenopus breviflorus Benth (*Lagenaria breviflora* Robert) belongs to the family Cucurbitaceae, and predominantly found in the West African countries, especially-Nigeria. The seed of *Adenopus breviflorus* Benth (*Lagenaria breviflora* Robert) was useful in folkloric medicine as an abortifacient and had been experimented to demonstrate anti-implantation activity in virgin female albino rats [4]. Gastrointestinal administration of the seed for the abortifacient and anti-progestational effects had been locally known, without side effects unlike when synthetic (OTC) contraceptive drugs were used for controlling female fertility. There are sparse information on the antifertility effects of the *Adenopus breviflorus* Benth seed. The present study was experimented to validate the folkloric evidence on the abortifacient potentials and acute toxicity (LD₅₀) of *Adenopus breviflorus* Benth seed.

2. Materials and methods

2.1. Collection of plant sample

Adenopus breviflorus Benth leaves and pods were collected from a domestic garden in Akure, Ondo State, Nigeria during sunrise and aerated until extraction.

2.2. Preparation of *Adenopus breviflorus* Benth Seed Extract

The method used for the preparation of aqueous-acetone extract of *Adenopus breviflorus* Benth seed was described thus, the leaves and tendril were separated from the pod and both were discarded, and the pod was broken by improvised mechanical press. Mixture was then preserved inside an air tight polythene bag to soften the endocarp and allow easy expulsion of the seed from the pod. The seeds were washed with water to remove slippery components and oven dried at 40 °C for 72 h to a constant weight. The dried seeds were then pulverized using Beltone Luinohun Blender/Miller III (model MS-223, Taipei, Taiwan). The powdered material was stocked in a sealed plastic container from which 1000 g was mixed with 1.0 L of distilled water:acetone (80:20) and stirred for 48 h at room temperature. This was then filtered with a sieve of considerable pore size. The filtrate was concentrated using rotary evaporator and freeze drying machines to give dried residue (brownish black caked).

2.2.1. Phytochemical screening

The presence of various plant constituents in the seed extract was determined by preliminary phytochemical screening [5].

2.3. GC–MS characterisation

The content was concentrated to 1 mL for gas chromatography analysis and 1 µl injected into the injection port of GC. The GC equipment used was HP 6890 powered with HP chemstation Rev. A09.01 (1206) software. The split ratio will be 20:1, the carrier gas was nitrogen at inlet temperature of 250 °C with a column type of HP INNOWax and column dimensions of 30 m x0.25 mm x0.25 µm. The oven program parameters include initial temperature at 60 °C, first ramping at 12 °C/min for 20 min, maintain for 2 min and second ramping at 15 °C/min for 3 min, maintained for 8 min. The detector used FID at 320 °C at hydrogen pressure 22 psi and compressed air of 35 psi.

2.3.1. Animals

Adult female Wistar albino rats, weighing 210–230 g were received from experimental Animal Care Center (University of Ilorin, Kwara State, Nigeria). All animals were maintained under controlled conditions of temperature (22 ± 1 °C), humidity (50–55 %) and light (12 h light/12 h dark cycle). They were acclimatized to the laboratory conditions for 14 days before the start of the experiment. Animals had free access to rat chow and drinking water. All experimental procedures were conducted in accordance with the Ethical Regulation and Guide for the Care and Use of Laboratory Animals [6].

2.3.2. Acute toxicity study

Healthy female albino rats were deprived of food and water for 4 h and subjected to lethal dose at 50 % (LD₅₀) and acute toxicity studies as described by Lorke, (1983) [7]. They were divided into 6 groups of 5 animals each and kept in separate cages during the experiment. The control group received food and water *ad libitum*. Groups 2–6 received suspension of extract of *Adenopus breviflorus* Benth seed orally at the doses of 10, 100, 1000, 1600, 2900 and 5000 mg/kg BWT daily. The rats were observed initially for 2 h for autonomic, behavioral and neurological changes, and for another 24 h to determine the LD₅₀.

2.3.3. Experimental design of the abortifacient activity

The plant extract was tested in female albino rats for abortifacient activity as designed and reported by Khanna et al. [8]. The female rats in proestrous phase were caged with males of proven fertility in the ratio of 2:1, in the evening and examined the following day for the evidence of copulation. Female rats exhibiting thick clump of spermatozoa in their vaginal smear were separated and that day was designated as Day 1 of pregnancy. These rats were randomly distributed into 4 groups, Control group and 3 Experimental groups of 6 animals each. On Day 10 of pregnancy, animals were laprotomised under light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers. The extract to be tested was then fed to confirmed pregnant rats, at doses of 100 (Group 2), 1000 (Group 3) and 1600 (Group 4) mg/kg body weight once daily by an intragastric (i. g.) soft rubber catheter from Day 11 up to the 15th day of pregnancy. The animals were allowed to go full term. After delivery the pups were counted and the abortifacient activity of extract was evaluated.

The following parameters were computed:

Number of live and dead fetuses;

$$\% \text{ survival ratio} = (\text{number of live fetus} / \text{number of live + dead fetus}) \times 100;$$

$$\% \text{ aborted} = (\text{number of aborted fetus} / \text{number of live + dead fetus}) \times 100;$$

$$\text{Resorption index} = (\text{total number of resorption sites} / \text{total number of implantation sites}) \times 100;$$

$$\text{Pre implantation loss} = (\text{number of corpora lutea} - \text{number of implantations} / \text{Number of corpora lutea}) \times 100;$$

$$\text{Post-implantation loss} = (\text{number of implantations} - \text{number of live fetuses} / \text{number of implantations}) \times 100.$$

The anogenital distance (AGD) and crown rump length (CRL) of litters were measured by using a measuring tape.

The variations in birth weight of litters and gestation period between control and experimental animal were also determined to check the abortive effect of *Adenopus breviflorus* Benth seed [9].

2.4. Tissues preparation

2.4.1. Effect on body weight and reproductive organ weight

Animals in each group were completely anaesthetized and then sacrificed by cervical decapitation. The ovary and uterus were carefully removed and weighed using digital electronic balance. Blood samples

were collected via cardiac puncture into non-anticoagulant tubes. The uteri and ovaries were rinsed in ice-cold 1.15 % potassium chloride solution and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) by using a Teflon homogenizer. The homogenized tissues were centrifuged at 3000g for 10 min at 4 °C [6].

2.4.2. Effect on hormonal levels

The sera of the control and experimental groups of female were analyzed for estrogen, progesterone, luteinizing and follicle stimulating hormone level with AccuLite master CLIA VAST Enabled kit.

2.4.3. MDA concentration in uteri tissue

A Thiobarbituric Acid Reactive Substances (TBARS) assay kit (Randox) was used to measure the lipid peroxidation product MDA equivalent. One hundred microliters of homogenate was mixed with 2.5 mL reaction buffer (provided by the kit) and heated at 95 °C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation product MDA levels are expressed in terms of nmoles MDA/mg protein using molar extinction coefficient of MDA- thiobarbituric chromophore (1.56×10^5 /M/cm).

2.4.4. GSH concentration in uteri tissue

The concentration of GSH was measured using standard laboratory method [10]. Homogenate was mixed with 0.2 M Tris buffer, pH 8.2 and 0.1 mL of 0.01 M Ellman's reagent, (5, 5'-dithiobis-(2-nitro-benzoic acid)) (DTNB). Each sample tube was centrifuged at 3000g at room temperature for 15 min. The absorbance of the clear supernatants was measured using spectrophotometer at 412 nm in one centimeter quartz cells.

2.4.5. GST activity in uteri tissue

The activity of GST was measured using standard laboratory method [11]. The reaction mixture consisted of 1.0 mM GSH, 1.0 mM CDNB, 0.1 M phosphate buffer (pH 7.4) and 0.1 mL of PMS in a total volume of 3.0 mL. The change in absorbance was recorded at 340 nm by using Shimadzu spectrophotometer UV-1601 and enzyme activity was calculated as nmol of CDNB conjugate formed $\text{min}^{-1} \text{mg}^{-1}$ protein using molar extinction coefficient of 9.6×10^3 /M/cm.

2.4.6. SOD activity in uteri tissue

The activity of SOD in cells was estimated using standard laboratory method [12], with the aid of nitroblue tetrazolium as the indicator. Superoxide anions are generated by the oxidation of hydroxylamine hydrochloride. The reduction of nitroblue tetrazolium to blue formazon mediated by superoxide anions was measured 560 nm under aerobic conditions. Addition of superoxide dismutase inhibits the reduction of nitroblue tetrazolium and the extent of inhibition is taken as a measure of enzyme activity. The SOD activity was expressed as units/mg protein as compared to a standard curve.

2.4.7. Total protein concentration in uteri tissue

This was carried out using the manufacturer protocol of Randox Total Protein Kit [13]. 1 mL of reagent R1(Sodium hydroxide (100 mmol/l), sodium-potassium tartrate (16 mmol/l), Potassium iodide (15 mmol/l) and copper II sulphate (6 mmol/l)) was added to 0.02 mL of the test sample, the mixture was incubated at 25 °C and the absorbance was then measured against the reagent blank at a wavelength of 546 nm.

Total Protein Concentration = (Abs Sample/ Abs Standard) x standard concentration

2.4.8. Statistical analysis

All values are expressed as mean \pm standard deviation. Statistical evaluation was done using One Way Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The significance level was set at $p < 0.05$.

3. Results and discussions

The quest for naturally occurring compounds from plants (phytochemicals) that could provide benefits as contraceptives and fertility control agents prompted the interest of this research. Meanwhile, folkloric belief as well as local uses of *Adenopus breviflorus* Benth seed as contraceptive had been reoccurring. This had been validated from the outcome of this study. Phytochemical screening of *Adenopus breviflorus* Benth seed revealed (Table 1) the presence of alkaloid, steroids, flavonoids, phenolics compound, saponins, and tannins. Reports had revealed that flavonoid isolated from *Striga lutea* and *Striga orabanchioides* have potent estrogenic and abortifacient effects [14,15], thus the presence of flavonoid could be responsible for infertility in female Wistar rats exposed to these plants [16] as well as *Adenopus breviflorus* Benth seed. The steroid in plants are precursors and could elevate the levels of sex hormones thus inducing impairment in fertility, the presence of steroid could be responsible for the abortifacient effect of the *Adenopus breviflorus* Benth seed, similar study was conducted on *L. trifoliata* leaves, in order to validate the antifertility potency of the leaf. Alkaloids suppresses the contraction of the uterine and impaired implantation, alkaloid is present in the seed extracts and could contribute to the anti-fertility potency exhibited by the seed extract. These alkaloids, steroids, flavonoids, saponins present in the *Adenopus breviflorus* Benth seed extract contributed via diverse mechanisms to the abortifacient activity.

Evaluation of acute toxicity revealed observable changes in behavioural, neurological and autonomic profiles as well as clinical symptoms such as salivation, respiratory distress, weight loss and change in appearance of hair at doses above 1600 mg/kg BWT, however there was no maternal mortality during the experiment. The LD₅₀ of the aqueous-acetone extract of the seed was greater than 5000 mg/kg BWT. This suggested that short term use at doses not greater than 1600 mg/kg BWT could be useful for abortifacient purpose and was apparently safe. The doses used for the abortifacient study were 100, 1000 and 16,000 mg/kg body weight. This finding was a little different from findings of Tajuddin et al. [17], while working on ethanolic extract of *Myristica fragrans* and Zade et al. [18] on *Moringa oleifera* in female rats.

The extract exhibited pregnancy interceptive activity which increased as the administered doses increased. Administration of 100 and 1000 mg/kg body weight (BWT) of the aqueous-acetone extract resulted in 20.31 % and 32.81 % abortifacient activity respectively, while administration of 1600 mg/kg BWT induced an abortifacient effect of 52.17 % (Table 2). This was evident from decrease in the percentage of live fetuses. This finding was similar to the antifertility

Table 1
Qualitative characterization of the phytochemicals in the aqueous-acetone extract of *Adenopus breviflorus* Benth seed.

Phytochemicals	Inferences
Alkaloids	++
Tannins	+
Phenolics	++
Glycosides	++
Saponins	+++
Flavonoids	++
Steroids	+
Terpenes	++
Anthraquinones	-
Chalcones	+

Table 2
Effect of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on fertility parameters.

Treatment Group	Dose (mg/Kg BWT)	Num. of foetus of rats on day 10	Num. of rats delivered (litter size)	Num. of resorptive in individual rats	Num. of resorption	Abortifacient activity (%)	Gestation period	Litter size	Litter BWT (gram)	AGD/CRL (mm)
Group 1	Vehicle (water)	14,10,11,12,13,11	14,10,11,12,13,11	0,0,0,0,0,0	0	0	22.12 ± 0.43a	6.8 ± 0.78d	5.1 ± 1.44c	1.13 ± 0.1b
Group 2	100 mg/kg BWT of extract	12,11,9,10,12,10	10,9,7,9,9,7	2,2,2,1,3,3	2.16 ± 0.753a	20.31	23.22 ± 1.88b	6.0 ± 1.11c	4.33 ± 1.82b	1.1 ± 0.25b
Group 3	1000 mg/kg BWT of extract	11,9,9,12,11,12	7,6,6,7,8,9	4,3,3,5,3,3	3.5 ± 0.837b	32.81	23.44 ± 1.03b	5.2 ± 0.33b	4.1 ± 1.66b	0.9 ± 0.11a
Group 4	1600 mg/kg BWT of extract	10,11,13,14,11,10	6,6,5,7,5,4	4,5,8,7,6,6	6 ± 1.414c	52.174	23.44 ± 1.44b	4.7 ± 0.54a	3.8 ± 1.81a	0.88 ± 0.135a

Values are expressed as mean ± S.E. for six albino rats in each group (P < 0.05). AGD: anogenital distance; CRL: crown rump length.

activities of *Ndigofera trifoliata* [3], the three varieties of *Ricinus communis* Linn [19] and reports by Yakubu et al. [20] using *Senna alata* leaves. There was a decrease in litter size with increase in the dose from 100 to 1600 mg/kg BWT of aqueous-acetone extract of *Adenopus breviflorus* Benth seed. The litter size of control group rats was the highest (6.8 ± 0.78) compared to the experimental groups 100 mg/kg BWT (6.0 ± 1.11), 1000 mg/kg BWT (5.2 ± 0.33) and 1600 mg/kg BWT (4.7 ± 0.54). The litter body weight in the control animal was 5.1 ± 1.44 g compared to the experimental groups which consistently reduced as the doses increased. The AGD/CRL ratio of the litter of rats dosed with *Adenopus breviflorus* Benth seed extract reduced significantly (p < 0.05) as the administered doses increased compared to the control group. This development could result from the failure of the embryonic development. The occurrence of foetal resorption (Table 3) predicted that the interruption of conception occurred after implantation of the foetus [21]. The weight of uterus and ovary significantly increased as the dose of aqueous-acetone extract of *Adenopus breviflorus* Benth seed increased. This suggested an induction of inflammation by aqueous-acetone extract of *Adenopus breviflorus* Benth seed on the ovarian and the uterine tissues, which was characterized by pain as well as malignant changes in the size of the tissues.

Scientific evidence had been provided on authentication of the abortifacient activity of series of plants including seeds of *Carica papaya*, *Garcinia kola* and leaves of *Bambusa vulgaris* and antifertility and abortifacient activities of phenolics, saponins and phytosteroids had been confirmed with sufficient data on animal models [2]. Effects of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on the concentration of serum reproductive hormones in pregnant female rats were shown in Table 4. When compared to the control group, administration of varying doses of the seed extract caused a significant reduction (P < 0.05) in the concentrations of LH, FSH, PH and EH. The hormone impairing activity of the extract corroborated its anti-gonadotropic effects. Similar outcomes were reported in studies on *Milletia aboensis* leaf [2] and *Indigofera trifoliata* [3] leaf. These confirmed that *Adenopus breviflorus* Benth seed had abortifacient potency. Quantitative determination of reproductive hormones in females can be used to diagnose ectopic pregnancy, ovulation characterizing luteal phase defect, conception and fertility. Follicle stimulating hormone (FSH) is a hormone which is secreted by the pituitary gland of the brain and it functions to promote the development of ova in females [22]. The significant reduction in FSH concentration by the extract administration depicted defect of the gonads which may inhibit folliculogenesis and delayed maturation of the follicles in the preovulatory phase. The extract could have exerted its effect on the anterior pituitary or hypothalamus since the release of FSH was regulated by gonadotrophic releasing hormone,

Table 3

Effects of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on the ovary, resorptive index and the weight of the reproductive organs.

Treatment Group	Corpora lutea (mean)	Fetuses resorption index (%)	Weight of uterus (mg)	Weight of ovary (mg)
Group 1 (Control)	20	0	121.4 ± 2.33 ^a	48.4 ± 2.11 ^a
Group 2 (100 mg/kg BWT of extract)	19	11.40	132.6 ± 1.96 ^b	51.25 ± 3.17 ^b
Group 3 (1000 mg/kg BWT of extract)	19	18.42	144.9 ± 3.44 ^c	53.88 ± 2.31 ^c
Group 4 (1600 mg/kg BWT of extract)	18	33.33	159.2 ± 1.18 ^d	57.19 ± 3.64 ^d

Values are expressed as mean ± S.E. for six albino rats in each group (P < 0.05).

Table 4Effects of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on fertility hormones in rats.

Treatment Group	LH mIU/mL	FSH mIU/mL	PH ng/mL	EH pg/mL
Group 1 (Control)	0.22 ± 0.001 ^d	0.192 ± 0.031 ^d	39.18 ± 2.17 ^c	75.11 ± 2.09 ^d
Group 2 (100 mg/kg BWT of extract)	0.18 ± 0.02 ^c	0.178 ± 0.011 ^c	37.9 ± 1.38 ^b	64.18 ± 1.27 ^c
Group 3 (1000 mg/kg BWT of extract)	0.13 ± 0.006 ^b	0.144 ± 0.038 ^b	32.66 ± 3.19 ^a	58.55 ± 2.37 ^b
Group 4 (1600 mg/kg BWT of extract)	0.09 ± 0.003 ^a	0.125 ± 0.077 ^a	32.1 ± 1.47 ^a	51.31 ± 1.43 ^a

Values are expressed as mean ± S.E. for six albino rats in each group (P < 0.05).

which adversely affected the capacity of the animal to conceive fetus [22] or the extract could impair the FSH signaling cascade in the gonad.

Luteinizing hormone (LH) is required for continued development and functioning of the corpora lutea as ovulation during the pro-oestrous stage. The significant reduction (P < 0.05) in the serum concentration of LH in the seed aqueous-acetone extract treated animals could be explained with physiological process of luteolysis preceding parturition or luteal phase that is not maintained [2]. The ability of the extract to inhibit LH inferred its ability to disrupt ovulation.

Table 5 revealed the effects of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on oxidative stress markers in the uteri tissues of the female rats. The treatment with the extract caused a significant increase in level of oxidative stress on the animal as revealed by the concentration of TBARS, T-PROTEIN and GSH and activities of GST and SOD in the uteri milieu (P < 0.05). The extracts significantly reduced the concentration of GSH and T-PROTEIN as the doses of the extract increase. The extract reduced the concentration of TBARS at 100 mg/kg body weight of rat without significant difference compared to the control (P < 0.05), followed by a significant increase in the concentration of TBARS as the dose increase to 1000 mg/kg and 1600 mg/kg body weight (P < 0.05). Treatment at all the doses reduced the activities of GST and SOD significantly. These connoted that these doses can pose deleterious damage to tissues disregarding the oral acute toxicity outcome which showed that it is not lethal at doses as high as 5000 mg/kg body weight of animal.

Plates 1–4: Uteri architectural distortion due to induced infertility by *Adenopus breviflorus* Benth seed.

Plates 1–4 and Table 6 revealed the uteri tissue architectural distortion caused by the treatment with 100, 1000 and 1600 mg/kg body weight of animals. There was an evident ovarian stroma with dense connective tissue in group treated with 100 mg/kg BWT, meanwhile, treatment with 1000 mg/kg BWT revealed ovarian tissue with sparse connective tissue and seen infundibulum, and treatment with 1600 mg/kg BWT did not reveal lesions, it had largely developing follicles but few matured tissues.

The GC–MS characterization of the seed was summarized in Table 7, with benzene 1,2,4-trimethyl (pseudocumene) (5.85 g/100 g of sample), mesitylene (1,3,5-trimethylbenzene) (4.28 g/100 g of sample) and isopropyl linoleate (4.27 g/100 g of sample) were predominant in the seed extract. Mesitylene and pseudocumene are isometric forms of benzene and components of many petroleum products. Study was conducted on the effects of mesitylene and pseudocumene vapours on pregnant Spague-Dawley rats during embryonic and fetal periods, however, the findings from the study revealed that the olfactory exposure of the isomeric derivatives of benzene did not cause embryolethality or teratogenicity, maternal toxicity was only evident by decrease in body weight as well as fetal weight decrease [23].

Table 5Effect of aqueous-acetone extract of *Adenopus breviflorus* Benth seed on antioxidant markers in rats.

Treatment Group	TBARS nM/mg Protein	GSH nM/mg Protein	GST nM/mg Protein	SOD U/mg/Protein	T-PROTEIN mg/dl
Group 1 (Control)	5.27 ± 1.29 ^a	8.83 ± 1.92 ^c	8.28 ± 1.19 ^d	6.55 ± 1.09 ^c	17.05 ± 0.18d
Group 2 (100 mg/kg BWT of extract)	5.11 ± 1.11 ^a	8.55 ± 2.88 ^c	6.33 ± 0.99 ^c	4.11 ± 0.876 ^b	12.83 ± 0.39 ^c
Group 3 (1000 mg/kg BWT of extract)	7.87 ± 1.89 ^b	5.19 ± 0.92 ^b	4.16 ± 1.22 ^b	4.0 ± 0.833 ^b	8.36 ± 0.66 ^b
Group 4 (1600 mg/kg BWT of extract)	9.27 ± 1.65 ^c	4.75 ± 1.28 ^a	3.21 ± 1.56 ^a	3.22 ± 0.911 ^a	5.49 ± 0.22 ^a

Values are expressed as mean ± S.E. for six albino rats in each group (P < 0.05).

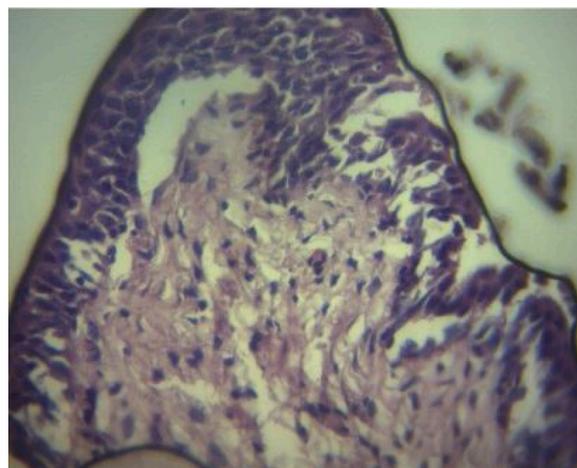


Plate 1. Group one (1) uteri tissue histology.

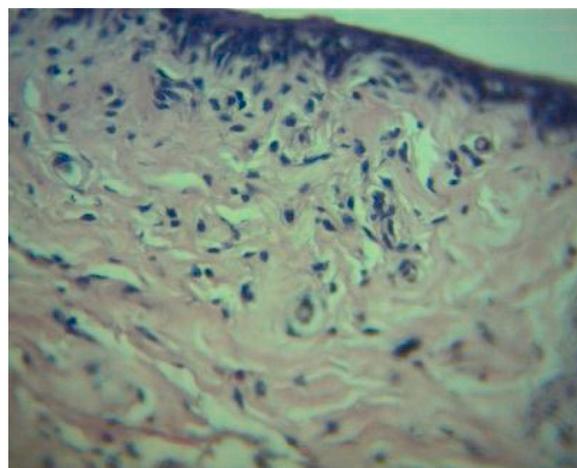


Plate 2. Group two (2) uteri tissue histology.

Trimethylbenzene had been reported to be able to reach the fetus [24]. The abortifacient potency of *Adenopus breviflorus* Benth seed hanged on these two phytochemicals which were revealed to be present in significant amounts in the seed.

In conclusion, the abortifacient activity lend support to the folkloric usage of *Adenopus breviflorus* Benth seed as an abortive agent, however,

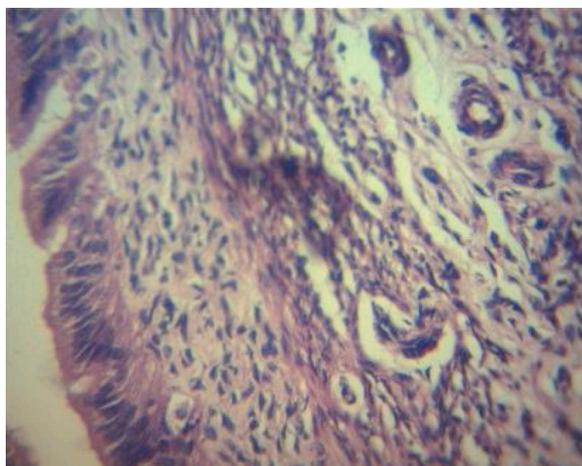


Plate 3. Group three (3) uteri tissue histology.

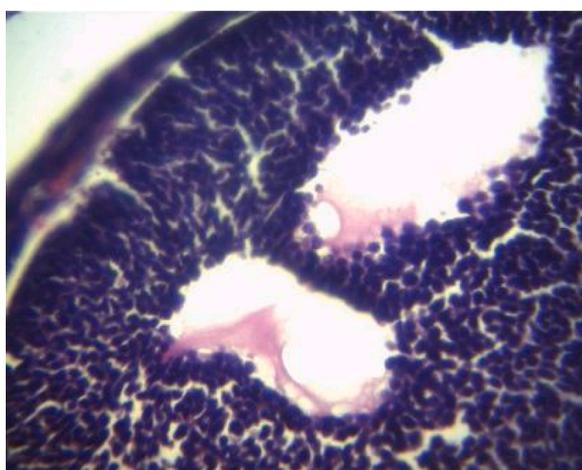


Plate 4. Group four (4) uteri tissue histology.

Table 6

Tabulated summary of the histology of the uteri tissues.

Group 1	Abundant connective tissue and myometrium seen (tubular tissue). No follicle seen.
Group 2	No developing follicle seen. The ovarian stroma has dense connective tissue
Group 3	The infundibulum of the uterine tubes seen, the ovarian tissue is very sparse
Group 4	No visible lesions seen. Large developing follicles seen, with few matured follicles.

Table 7

GC–MS analyses of aqueous-acetone extract of *Adenopus breviflorus* Benth seed.

	RT	Area %	Library ID	Ref#	CAS#	Qual
1	7.115	4.28	Mesitylene	9399	000108-67-8	95
2	7.739	5.85	Benzene, 1,2,4-trimethyl	9421	000095-63-6	95
3	8.294	3.5	Decane	19157	000124-18-5	87
4	33.825	2.86	n-hexadecanoic acid	107547	000057-10-3	95
5	42.528	4.27	Isopropyl linoleate	162975	022882-95-7	76

bioactive principles of abortifacient effect had been justified and the

presence of mesitylene and pseudocumene had been implicated in this effect. This study has established an effective and safe remedy for contraceptive application of aqueous-acetone extract of *Adenopus breviflorus* Benth seed.

Declaration of Competing Interest

The authors declare no conflict of interest.

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