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Phytochemical Screening and Contractile Activity of Methanol Extracts of *Acanthus montanus* on Albino Rat Uterus

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Authors' contributions

This work was carried out in collaboration between all authors. Author OJO designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors OI and MEJ managed the analyses of the study. Author MEJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the chemical and uterine contratile activity of extract of *A. montanus* using the Ugo basile organ bath model 4050.
Study Design: Five female Wistar non-pregnant rats weighing between 160-200 g were brought into oestrus by injecting 0.2 mg/kg of stilbesterol in ethanol intra-peritoneneally for 24 hours. The rats were sacrificed under chloroform anaeshesia. The uterine horns were identified and the two horns of the uterus cut out and transferred to a petri-dish containing physiological salt solution (PSS). 1.0-1.5 cm length was mounted in a 10 ml organ bath containing De-Jalon solution with the following chemical compositions NaCl, 154mM/L $NaHCO_3$, 5.95 mM/L, D-Glucose, 2.78 mM/L KCl, 5.40 mM/L,$CaCl_2.2H_2O$, 5.44 mM/L. The tissue was aerated with air via an aerator and the temperature maintained at 37ºC with a pH of 7.4. The spontaneous contractions of the uterus were recorded by means of force displacement transducer connected to an amplifier and a multi-channel

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recorder and allowed to equilibrate for 30 minutes with periodic changing of the bath fluid every ten minutes under a resting tension of 750 mg.
The effect of the extract on the concentration response curves for oxytocin and acetylcholine was determined. Hence responses to different doses of oxytocin and acetylcholine alone and in the presence of the extract were also obtained. The effects of two positive controls (Salbutamol and atropine) were also determined.
The experiment was repeated five times and the mean and standard deviation of the results were calculated.
Place and Duration of Study: Department of Chemistry and Pharmacology, University of Benin, Benin City, between March 2016 to July 2017.
Methodology: A total of 980 g of the plant material was extracted with a soxhlet apparatus using 1.5 L of methanol (BDH, England) as solvent. The extract was concentrated in vacuo at 50ºC. The extract was stored in a refrigerator at 4ºC until needed for the experiment.
Female non-pregnant Wistar rats were pre-treated intra-peritoneally with 0.2 mg/kg of diethylstilbestrol 24 h prior to the actual experiment [1]. The rats were sacrificed under chloroform anaesthesia. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 10 mL organ bath containing De-Jalon physiological salt solution. The tissue was bubbled with air using an aerator and temperature was maintained at 37ºC, with a pH of 7.4. The spontaneous contraction of the uterus was recorded with 7003-B transducer connected to an Ugo Basile Data capsule device. The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg). The tissue was allowed to equilibrate for 30 minutes before the start of the experiment and placed under tension of 500 mg.
Results: From the phytochemical screening result, terpenoids was observed in larger amount than other phytochemicals. The presences of these bioactive constituents have been reported to have physiological effect in man [2].
The results also showed that various concentrations of oxytocin and acetylcholine produced a significant contraction of the rat uterus. Administration of the extract produced a significant (P = .05) dose-dependent reduction in oxytocin and acetylcholine induced contractions by the extract at concentration tested (Figs. 1-2). From the graphical plot, the percentage response to log dose of *Acanthus montanus* extracts supplemented with oxytocin exhibited anti-contractile effect of the uterus of rats at 30%: -2.699, 40%: -2.398 and 45%: -2.097 (Fig. 1), while for Acetylcholine induced contraction, the effect of anti contractile activity was observed at 25%: -0.699, 40%: -0.398, 45%:-0.097 and 45%: 0.301 (Fig. 2). This study revealed that extract at both doses produced significant inhibition of oxytocin and acetylcholine induced contractions of the uterine smooth muscle in non-pregnant rats.
Conclusion: The methanol extract of *Acanthus montanus* was found to possess anti-contractile activity on the uterine smooth muscles in non-pregnant rats, which corroborates the use of the plant extract in the treatment of spontaneous abortion.

Keywords: Acanthus montanus; extraction; uterine contractile activity; phytochemicals.

1. INTRODUCTION

Acanthus montanus (Nees) T. Anders, family-Acanthaceae is one of the medical plants used to facilitate birth among traditional healers in Edo State, Nigeria. Medicinal plants like *Phyla nodiflora* (L) [3] *Aleurites moluccana* (L) [4] and *Newbouldia lavis* [5] have been reported to stimulate uterine contraction in rats. Some plants have been reported to possess tocolytic activity such as *Pyrenacantha staudtii* [6,7 and 8] While those inducing abortion includes *Caesalpinia pulcherrima*, *Gossyplum harbaceum* [9] and Others controlling spontaneous abortion (miscarriage) include *Zehneria scabra*, *Sida urens*, *Rhipslis Cassytha* [10] and *Aframomum melegueta*.

Acanthus montanus in Nigeria is locally called cogwudurunwashishi' (Ibo), ebe-igbe'(Akoko-Edo) 'karinkan' (Yoruba) and odi-igban (ijaws) [11]. The plant is a thinly branched perennial with basal clusters of oblong to lance-shaped glossy, dark green leaves reaching up to 12 inches (30 cm) long. The leaves have silver marks and wavy margins. It reaches up to 6 feet (1.8m) tall and about 24 inches (61cm) wide. It's a shrub widespread in Africa, the Balkans, Romania, Greece and Eastern Mediterranean [12].

The 'okpameri' people in Edo State of Nigeria use the soup of the plant leaves and the young twigs for abdominal pains caused by indigestion [11]. According to [13] and [11] the aqueous extract of the plant is used for the traditional treatment of pain, female infertility and spontaneous abortion with dosage schedule depending on the ailment.

In Africa alone, the statistics of miscarriages (spontaneous abortion) whether natural or induced are 2.4 million (eastern Africa); 930,000 (middle Africa); 1.8 million (western Africa) [14]. In Nigeria, about 10-20% of all pregnancies end as miscarriages [2]. Thus, this research is aimed at determining the uterine relaxant or contractile activity of *Acanthus mantanus* in non pregnant rat in order to reveal the pharmacological importance of the plant and add to scientific knowledge on the verified ethno medicinal use of the plant.

2. EXPERIMENTAL DETAILS

2.1 Plant Collection and Extraction

Fresh leaves of *A. montanus* were collected from their natural habitat in Egor Local Government Area of Edo State, Nigeria. The plant was identified by Dr. E.I. Aigbokhan, a taxonomist in the Department of Plant Biology and Biotechnology, University of Benin, Benin City. The leaves were washed with caution and air-dried in the laboratory for four weeks and pulverized to a fine powder.

Four hundred and twenty grammes (420 g) of the sample was extracted with 99.5% methanol (NDH, England) in a soxhlet extractor for 8 hours using 50 g at each batch. The extract was stored in sample bottles in desiccators for further analysis.

2.2 Phytochemical Screening of Plant Extracts

Phytochemical screening of glycosides, saponins, phenolics, flavonoids, tannins, eugenols, terpeniods, steroids and alkaloids were performed by standard procedures according to [15] and [1].

2.2.1 Test for glycoside

1ml of the extract was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was under - laid with 1 ml of conc. H_2SO_4. A brown ring is required for the presence of glycoside.

2.2.2 Test for saponin

0.5g of the extract was shaken with water in a test tube and observed for frothing. Saponin rein Weiss (supplied by Merck) was use as Standard.

2.2.3 Test for flavonoids

2 ml of the extract was boiled in 10ml of distilled water and filtered. The filtrate was divided into two portions A and B of 5 ml each:

To portion A: 10% lead acetate solution was added in few drops. A yellow precipitate is indicative of positive result.

To portion B: 5 ml of 20% NaOH and few drops of dilute HCl were added to the solution, formation of a colourless solution is indicative of a positive test.

2.2.4 Test for phenolic compounds

1ml of extract was added to 5 ml of 90% ethanol. In addition, 1 drop of 10% $FeCl_3$ was added. A pale yellow colouration is indicative of positive test.

2.2.5 Test for tannins

To 2 ml of the extract, 10 ml of distilled water was added and boiled for 5min. and then filtered into two halves.

i. To about 2 drops of the filtrate, ferric chloride ($FeCl_3$) solution was added; formation of a bluish precipitate is required for hydrolysable tannin.
ii. To about 5 drops of the filtrate, 2 ml dilute HCl was added and boiled for 5 min. Red precipitate is required for the presence of tannin.

2.2.6 Test for eugenol

2 ml of the extract was mixed with 5ml of 5% KOH solution. The aqueous layer was separated and filtered. Few drops of dilute HCl were added to the filtrate. A yellow precipitate is indicative of positive test.

2.2.7 Test for steroids

2ml of acetic anhydride was added to 0.5g plant extract in 2ml of dilute H_2SO_4. A colour change

from violet to blue or green is required for the presence of steroids.

2.2.8 Test for terpenoids

The extracts of the plant materials were taken in a clean test tube, 2 ml of chloroform were added and it was vigorously shaken, then evaporated to dryness, 2 ml of concentrated sulphuric acid was then added and the mixture heated for about 2 minutes.

2.2.9 Test for alkaloids

Drangendoff's Wagner's reagent and picric acid were used to test for alkaloids. About 1 ml each of the plant extract transferred into different test tubes labeled A, B and C.

To portion A: 2 mls of dragndoff's reagent (made of a mixture of potassium Bismuth iodide salt) was added. Reddish brown precipitate is required for a positive test.

To portion B: 2 mls of wagnger's reagent was added. Reddish brown precipitate is indicative of a positive test.

To portion C: 2 mls of picric acid was added to the plant extract. A yellow precipitate is a positive test.

2.3 Animals

Non-pregnant female albino rats were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were maintained under standard environmental conditions and had free access to standard rat feeds (Broiler mash) and water. The rats were allowed acclimatized periods of two weeks before the experiment. The animals were handled according to standard guidelines for the use and care for experimental animals.

2.4 Physiological Salt Solution (De-Jalon)

NaCl (45.0 g), KCl (2.10 g), $NaHCO_3$ (2.50 g) and D-glucose (2.50 g) were weighed and made into a solution of 3.5 L distilled water. Calcium chloride (0.40 g) was made into a solution of 1.5 L distilled water in a separate beaker. Both solutions were then added to give 5 L.

2.5 Acute Toxicity Test

The acute toxicity of *A. montanus* in rat (n= 20) was estimated using the method described by [16]. Animals received oral administration of 10, 100, 1000 and 5000 mg/kg of methanol extract of *A. montanus*. Control group received distilled water orally. Animals were observed for 24 hours for death and other toxic signs/symptoms

2.6 Pharmacological Screening

Female non-pregnant Wistar rats were pretreated intraperitoneally with 0.2 mg/kg of diethyl/stilbestrol 24 h prior to the determination of the contractile activity [17]. The rats were sacrificed under chloroform anaesthesia. The abdomen was opened and the two horns of the uterus carefully isolated, freed of mesenteric fat and a 1 cm piece was mounted in a 10 mL organ bath containing De-Jalon physiological salt solution. The tissue was bubbled with air using an aerator and temperature was maintained at 37ºC, with a pH of 7.4. The spontaneous contraction of the uterus was recorded with 7003-B transducer connected to an Ugo Basile Data capsule calibrated channel recorder (model: 4050). The transducer was previously calibrated to establish a relationship between the force applied to the transducer and the gauge deflection (500 mg). The tissue was allowed to equilibrate for 30 min before the start of the experiment and placed under tension of 500 mg.

The concentration-response curves of oxytocin and acetylcholine induced contractions were first obtained and this concentration response curves each were repeated in the presence of the extract (0.1, 0.2 mg/ml and 0.5 mg/ml) to investigate its effect.

The effect of the extract was compared to two positive controls (salbutamol and atropine) for oxytocin and acetylcholine concentration response curves respectively.

3. RESULTS AND DISCUSSION

The results of the phytochemical constituents and contractile activity on non-pregnant rat of *A. montanus* are shown in Table 1.

Terpenoids was observed in larger amounts than other phytochemicals. However, flavonoids, phenolics and tannins were not detected. The presences of these bioactive constituents have

been reported to have physiological effect in man [2].

Table 1. Phytochemical constituents in methanol extract of *A. montanus*

Serial number	Phytochemical constituents	Methanol extract
1	Glycosides	+
2	Saponin	+
3	Flavonoid	-
4	Phenolics	-
5	Tannins	+
6	Eugenol	+
7	Steroid	+
8	Terpenoid	++
9	Alkaloid	+

+=present - = absent ++ = largely present

3.1 Uterine Effect

The results of the effect of methanol extract of *Acanthus montanus* on oxytocin and acetylcholine induced contraction in the non-pregnant rat uterus are shown on Figs. 1 and 2.

The results showed that various concentrations of oxytocin and acetylcholine produced a significant contraction of the rat uterus.

Administration of the extract produced a significant (P<0 .05) dose-dependent reduction in oxytocin and acetylcholine induced contractions by the extract at concentration tested (Figs. 1-2). From the graphical plot, the percentage response to log dose of *Acanthus montanus* extracts supplemented with oxytocin exhibited anti-contractile effect of the uterus of rats at 30%: -2.699, 40%: -2.398 and 45%: -2.097 (Fig. 1),

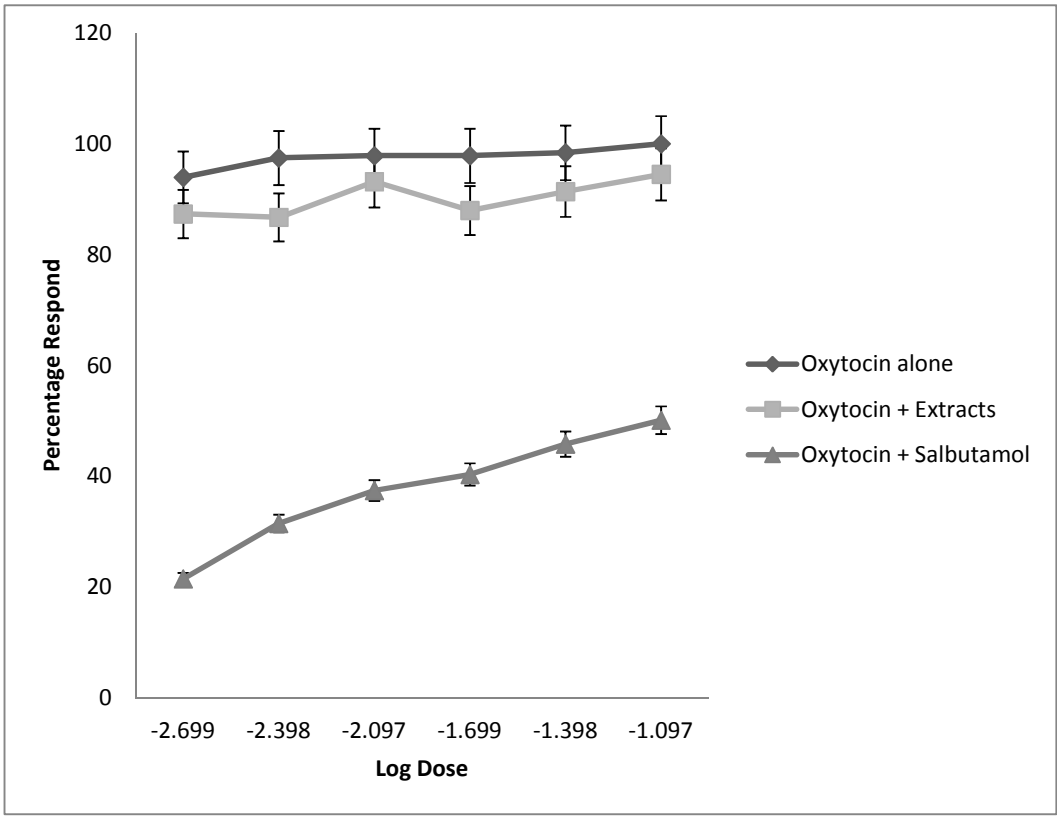

Fig. 1. Effects of the methanol extract of *Acanthus montanus* on oxytocin induced contraction in the non-pregnant rat uterus

*Values are mean percentage response ± SEM (n= 5 per group). *P 0 =.05 significantly different from oxytocin induced contraction alone.*
OXY: Oxytocin alone
OXY+: Oxytocin and 0.1 m/100 mg/ml of Acanthus montanus
OXY+Sal: Oxytocin and 5 µg of Salbutamol

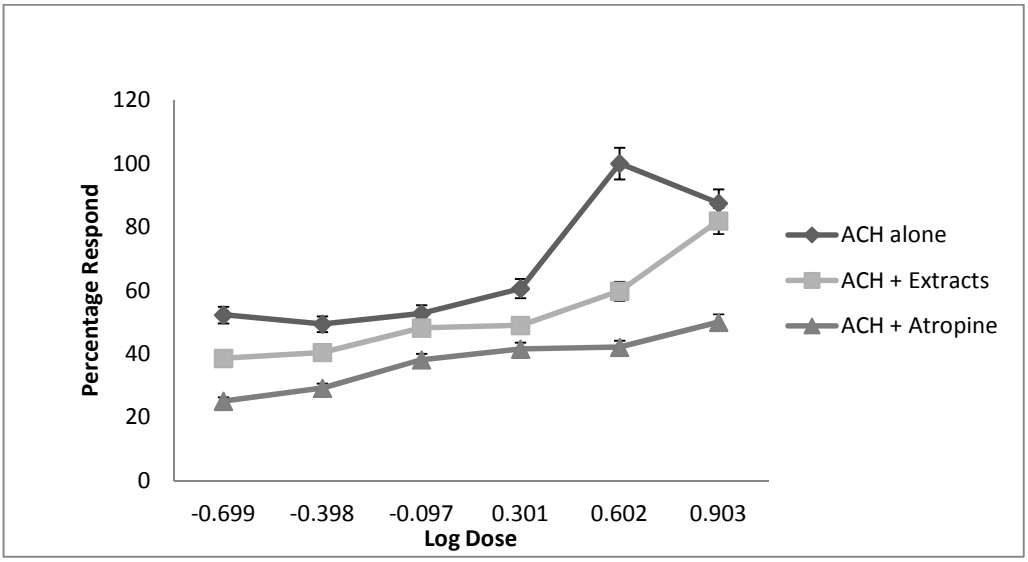

Fig. 2. Effects of the methanol extract of *Acanthus montanus* on acetylcholine induced contraction in the non-pregnant rat uterus

*Values are mean percentage response ± SEM (n= 5 per group). *P<.05 significantly different from acetylcholine induced contraction alone*
ACH: acetylcholine alone
ACH: acetylcholine and 0.1 m/100mg/ml of Acanthus montanus
ACH +Atr: Acetylcholine and 5 µg of Atropine

while for acetylcholine induced contraction, the effect of anti contractile activity was observed at 25%: -0.699, 40%: -0.398, 45%: -0.097 and 45%: 0.301(Fig. 2). This study revealed that extract at both doses produced significant inhibition of oxytocin and acetylcholine induced contractions of the uterine smooth muscle in non-pregnant rats. The work of Newal [18] indicated also that the effect on acetylcholine induced contractions may be due to muscarinic receptor blockade. These results also indicate a shift of the concentration response curve to the right produced by both doses of the extracts [19 and 20].

3.2 Acute Toxicity

The absence of death at 5000 mg/kg of the extract shows that the lethal dose of the methanol extract of the plant is higher than 5000 mg/kg which may be an indication of the safety of the plant.

4. CONCLUSION

The methanol extract of *Acanthus montanus* was found to possess anti contractile activity on the uterine smooth muscles in non-pregnant rats, which corroborates the use of the plant extract in the treatment of spontaneous abortion.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee" and the approval number is ET/FP/018/25.

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COMPETING INTERESTS

Authors have declared that no competing interests exist

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