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Full Length Research Paper

Studies on the effects of the methanol extract of *Struchium sparganophora* (L.) Ktze (Asteraceae) on the rat uterus

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***Struchium sparganophora* (L.) Ktze (Asteraceae) is one of the vegetables equally consumed for medicinal purposes in the South Western part of Nigeria where it is used to treat a range of infections. The ethnomedical claimed use of the plant as an abortifacient crude drug was investigated on diethylstilboestrol treated rats. The methanol extract and its aqueous, and chloroform fractions were tested at the concentrations of 10 to 160 mg/ml corresponding to final bath concentrations (FBC) of 200 to 3200 µg/ml. The activities of the extract were also examined in the presence of oxytocin, acetylcholine, salbutamol and atropine. At a concentration of 10 mg/ml, the extract induced uterine contraction equivalent to 0.96 ± 0.23 g force which increased to 1.98 ± 0.07g at a concentration of 160 mg/ml. The aqueous fraction produced maximum contraction of 0.88 ± 0.33 g at the highest concentration while the chloroform fraction was inactive. While simultaneous administration of 0.008 µg/ml oxytocin or 0.004 µg/ml acetylcholine augmented the contractile effects of the extract, its activities were significantly attenuated in the presence of 1 µg salbutamol or 2 µg atropine. The results showed that the leaves of *S. sparganophora* have contractile effects on the uterus and partial separation of the constituents did not enhance the activities. The effects are probably mediated through the activation of the oxytocin and muscarinic receptors.**

Key words: *Struchium sparganophora*, methanol extract, uterine contraction.

INTRODUCTION

Medicinal plants provide alternative source of drugs to many people in various parts of the world where there are insufficient availability of orthodox health care facilities coupled with precarious drug distribution. Such plants are used to treat wide range of disease conditions ranging from physical to internal health abnormalities. Among medicinal plants found growing ubiquitously in the South Western part of Nigeria is *Struchium sparganophora* (L.) O. Ktze (Asteraceae). It is a shrub that occurs more commonly as a cultivated species than as a wild plant growing near the waterside (Oboh, 2006) and locally known as *Ewuro odo* in Yoruba. It is a vegetable usually used in the preparation of soup. In ethnomedicine, the plant was reportedly useful in the treatment of cutaneous and subcutaneous parasitic infections, diarrhea, dysentery as well as, venereal diseases. It is also useful as an abortifacient, that is, it can be used to induce the contraction of the uterus (Burkill, 1985). Furthermore, it is reportedly useful in the treatment of rheumatic pains (Olabanji et al., 1995). Recently, using the tadpoles of *Raniceps ranninus* and the radicles of *Sorghum bicolor* seed models, the claimed use of the plant in treating tumour related ailments was partly investigated (Ayinde and Agbakwuru, 2010). In furtherance of our work on this plant, we hereby, report our investigation on the probable effect of the leaf extract on the rat uterus with a view to

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ascertain the claimed use of the plant as an abortifacient crude drug.

MATERIALS AND METHODS

Collection and processing of the plant material

The fresh leaves of *S. spaganophora* were collected in September, 2009 in Iwo, Osun State Nigeria. The identity of the plant was confirmed by Dr. Shasanya Olufemi, the plant taxonomist at the Forest Research Institute of Nigeria (FRIN), Ibadan. A herbarium specimen number FHI 108438 was deposited at the Institute for reference. The plant material was air dried in the laboratory for five days at room temperature followed by oven drying at 40°C after which it was ground to powder form using an electric mill. The powdered sample was kept in air tight container until required.

Extraction of the plant material

About 500 g of the powdered leaves of *S. spaganophora* was macerated in 2.5 L of methanol for 72 h. After filtration and concentration using rotary evaporator maintained at 40°C, the extract obtained was weighed (22.4 g; 4.48%) and kept in the refrigerator.

Organic solvent partitioning of the methanol extract

About 11.9 g of the methanol extract was dissolved in 200 ml 50% aqueous methanol and was exhaustively partitioned using chloroform (3 × 75 ml) in a separating funnel. The chloroform and aqueous fractions obtained were concentrated using a rotator evaporator. The chloroform fraction weighed 4.5 g while the aqueous fraction weighed 6.0 g.

Drugs and chemicals used

These include Oxytocin, Salbutamol (G. Richter), Atropine (Indus Pharma), D- glucose, potassium hydrogen phosphate, magnesium sulphate heptahydrate, calcium chloride dehydrate (Merck), sodium chloride (BDH Chemicals), sodium hydrogen carbonate (T. G. I. Ltd) and potassium chloride (Cambian chemicals).

Preparation of the animals

After obtaining permission from the Faculty of Pharmacy Committee on the use of animals for experiments, adult non-pregnant female albino rats (150 to 250 g) were obtained from the animal house of the Department of Pharmacology and Toxicology, University of Benin, Benin City, where they were maintained on normal animal pellets (Livestock, Benin) and water *ad libitum*. Each animal was pre-treated with 0.2 mg/kg diethylstilbestrol which was administered interperitonially 24 h before the experiment. The animals were later anaesthetized with diethyl ether and dissected to harvest the uterus. About 1.5 cm of the uterine horns were removed and cleaned free from fatty and mesenteric tissues. Each strip of the uterus was suspended in 50 ml organ bath containing De Jalon's solution composed of (mM): NaCl 154.1, $NaHCO_3$ 5.95, d-glucose 2.75, KCl 5.36 and $CaCl_2.2H_2O$ 0.055. The solution was maintained at 36 ± 1°C and aerated with 5% CO_2 in O_2.The uterine strips were connected to a single channel (Ugo Basile 7050) recorder via an isometric force displacement transducer (Ugo Basile) previously calibrated to establish the relationship between the force applied to the transducer and gauge deflection with a 500 mg corresponding weight with a sensitivity of 6. The preparations were allowed to equilibrate for 30 min before the administration of the extract or drugs.

Effects of the extract, the organic solvent fractions and the drugs on the uterus.

The effects of the methanol extract, the chloroform and the aqueous fractions were tested at concentrations of 10 to 160 mg/ml corresponding to final bath concentrations (FBC) of 200 to 3200 µg/ml. Each experiment was replicated five times.

Standard drugs used were Oxytocin and Acetylcholine which have uterotonic effects were administered for comparison of effect on the uterus. Oxytocin was administered at concentrations of 0.1 to 1.0 I.U (corresponding to 8×10^{-4} to 3.2×10^{-2} µg/ml FBC). Probable synergies or antagonisms between the extract, the aqueous and the chloroform fractions on one hand and oxytocin or acetylcholine were investigated while the effects of atropine and salbutamol on the activities of the extract were examined by pre-treating the tissues with 2 and 1 µg of the drugs respectively.

Statistical analysis

All data were expressed as mean ± SEM (standard error of mean) with n representing the number of animals used. Where applicable, the data were compared using one way analysis of variance (ANOVA), Graph pad InstantR version 2.05 a software (UK). The level of significance was from $P < 0.05$.

RESULTS AND DISCUSSION

The crude extract and the aqueous fraction of *S. sparganophora*, Oxytocin, Acetylcholine (Ach) were all observed to produce concentration dependent contractile effects on the primed isolated uterus. At a concentration of 200 µg/ml, the extract elicited a uterine contraction equivalent to 0.96 ± 0.23 g force which increased to 1.98 ± 0.07 g with the extract concentration of 3200 µg/ml. The aqueous fraction of this extract was observed to produce uterine contractions equivalent to 0.18 ± 0.05 g and 0.88 ± 0.33 g at concentrations of 200 and 3200 µg/ml respectively. The chloroform fraction was observed to produce no contractile effect on the uterus. The variations in the activities of the crude extract and the fractions organic solvent fractions were observed to be significant at $P < 0.001$ (Figure 1).

Oxytocin at a FBC of 0.0008 µg/ml produced a force of contraction of 0.86 ± 0.27 g which increased to 1.63 ± 0.267 g with a concentration of 0.008 µg/ml. A maximum contraction of 1.97 ± 0.12 g was obtained after administration of 0.032 µg/ml (Results not shown). Also, simultaneous administration of 0.008 µg/ml oxytocin with the various concentrations of the crude extract produced significantly ($P < 0.05$) enhanced contractile effects. For instance, a contraction of 0.96 ± 0.23 g produced by the extract alone at a concentration of 200 µg/ml was noted to be 1.97 ± 0.06 g in the presence of 0.008 µg/ml. Also, a contraction of 1.51 ± 0.17 g produced by the extract at

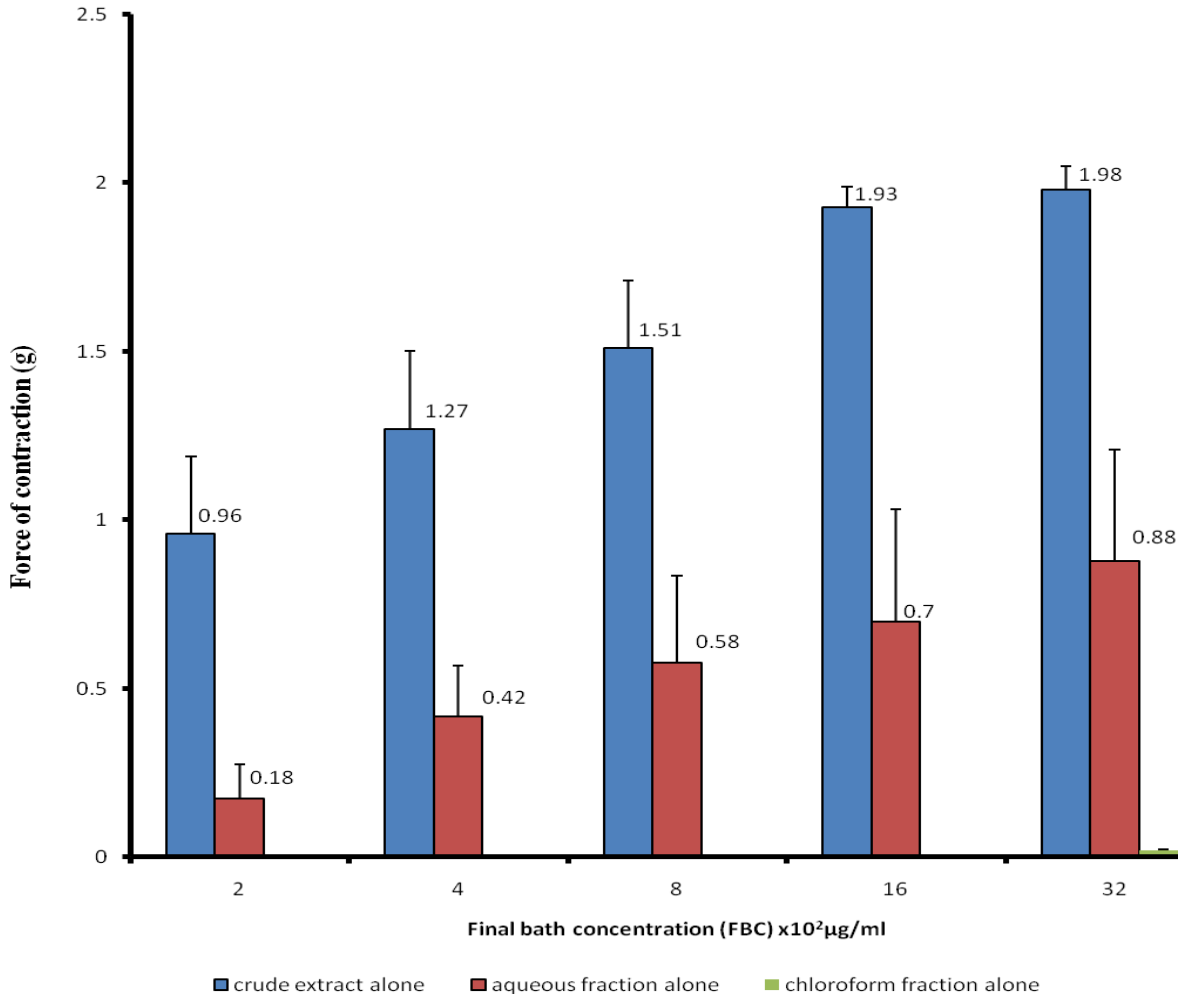

Figure 1. Contractile effect of crude extract and its organic solvent fraction on the rat uterus.

a concentration of 800 µg/ml increased to 2.03 ± 0.13 g in the presence of the oxytocin (Figure 2).

Acetylcholine at the concentration of 0.004 µg/ml elicited a corresponding force of 0.334 ± 0.09 g which again increased to a maximum of 1.98 ± 0.19 g with a concentration of 1.6 µg/ml (Results not shown). Also, simultaneous administration of 0.004 µg/ml acetylcholine with the various concentrations of the crude extract produced remarkable synergistic effects. Administration of 200 µg of the crude extract in the presence of 0.004 µg/ml acetylcholine produced a force of contraction of 2.08 ± 0.061 g as against 0.96 ± 0.23 g produced by the extract alone ($P < 0.001$) (Figure 3).

The pre-treatment of the uterine tissue with 2 µg atropine ten (10) min before the administration of various concentrations of the extract slightly but not significantly attenuated the contractile effects of the extract on the uterus. A contraction of 0.96 ± 0.23 g produced by the extract at a concentration of 200 µg /ml was reduced to 0.91± 0.21 g in the presence of the atropine. Also, the contraction of 1.93 ± 0.09 g produced by the extract at a concentration of 1600 µg /ml was reduced to 1.54 ± 0.19 g in the presence of the atropine. On the other hand, pre-treating the uterine tissue with 1 µg Salbutamol before administering the extract produced highly remarkable reduction in the contractions elicited by the extract. At lower concentrations of the extract, 1 µg salbutamol completely abolished the effects of the extract while the highest uterine contraction of 1.98 ± 0.07 g produced by 3200 µg/ml of the extract was reduced to 0.882 ± 0.15g in the presence of the drug (Figure 4). As the mild uterine contractile effects of the aqueous fraction were enhanced by the presence of either 0.008µg/ml oxytocin or 0.004 µg/ml acetylcholine, simultaneous administrations of increasing concentrations of the chloroform fraction with either of the drugs were observed to produce reduced contractile effects produced by either of these drugs alone. At lower concentrations of the two drugs, the chloroform fraction completely abolished the contractile effects of the drugs. However, at higher concentrations,

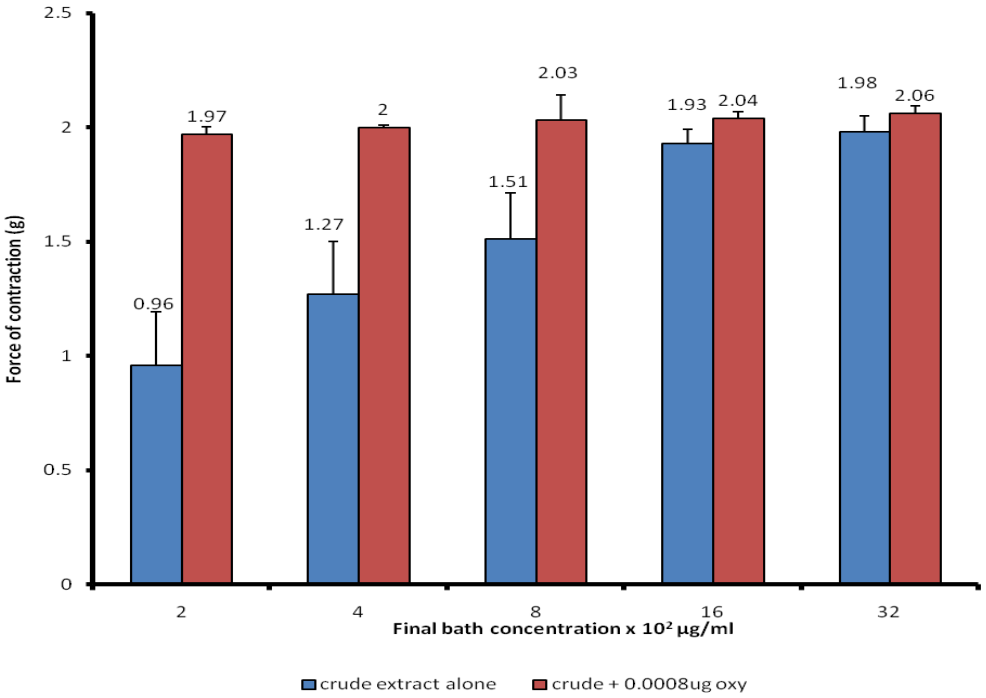

Figure 2. The effect of the 0.0008 µg oxytocin on the uterine contraction induced by the methanol extract.

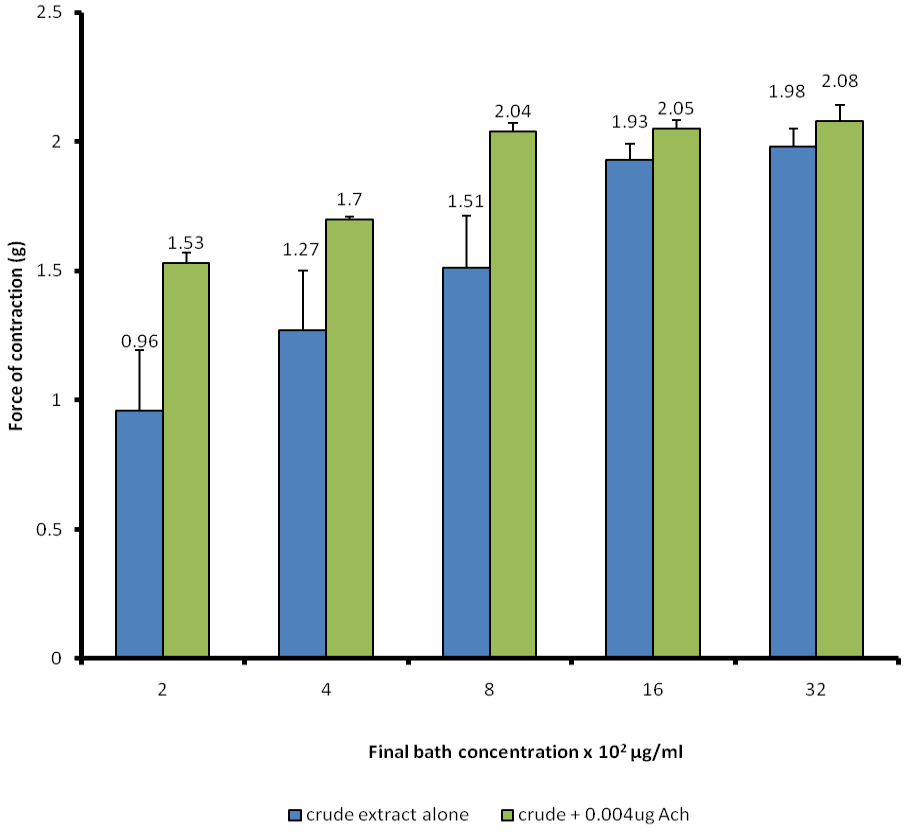

Figure 3. The effects of the 0.004 µg acetylcholine in the uterine contraction induced by the methanol extract.

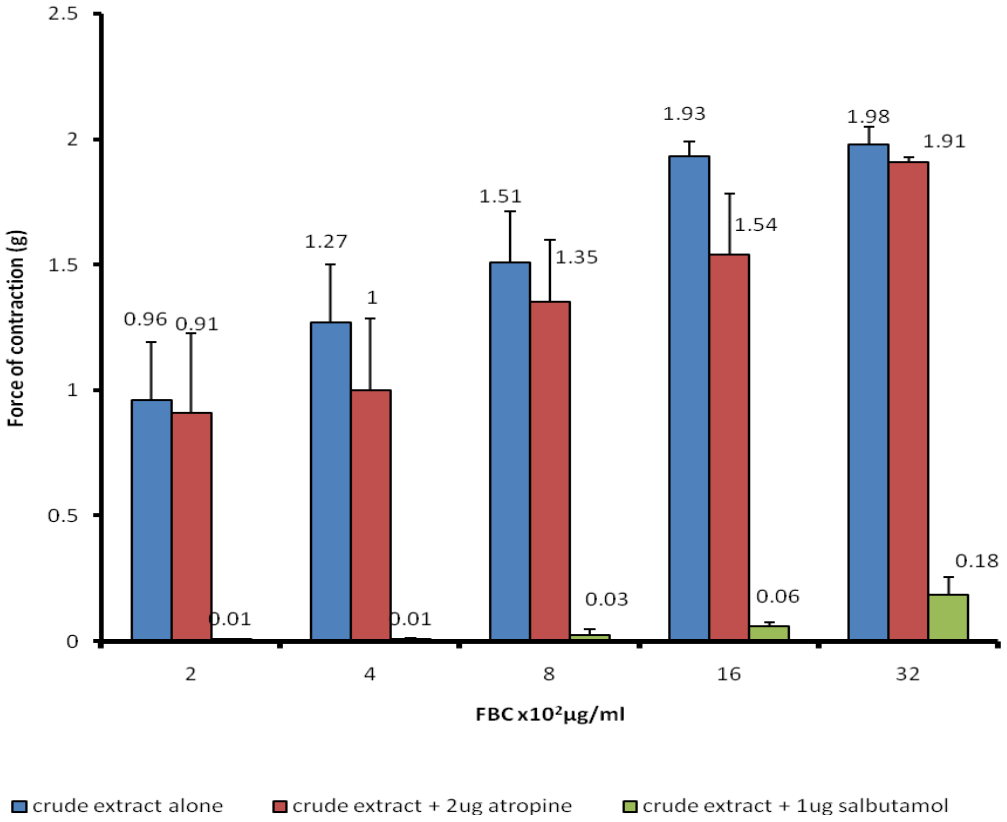

Figure 4. Effect of 2 µg atropine and 1 µg salbutamol on the uterune contraction induced by crude extract.

the contractions were restored but not as remarkable as either the oxytocin or the acetylcholine alone (Figures 5 and 6).

The physiological effects produced by medicinal plants on animal tissues are direct functions of the various secondary metabolites they contain. In our previous reports (Ayinde and Agbakwuru, 2010) *S. sparganophora* has been shown to contain tannins, flavonoids, saponins and alkaloids. The contractile effects exhibited by the crude extract could be due to any of the group of constituents. An attempt to situate the activity of the extract in either the chloroform soluble or aqueous fractions through partitioning revealed that the extract produced higher contraction than either the chloroform or the aqueous fractions. This observation implied a probable synergism in the activities of the plant constituents in effecting uterine contraction. Some of these constituents may be acting by potentiating the oxytocin receptors which resulted in increased contraction over what was observed with either the extract or the oxytocin alone. The extract may enhance the binding of oxytocin to the uterine tissues thereby, causing a greater response or vice versa.

Salbutamol is a known agonist of β- 2 receptors on the uterus where it causes relaxation of the uterine smooth muscles even in dysmenorrheic women (Lalos and Joelsson, 1987). As the contractile effect of the extract was completely abolished by the salbutamol, it can be inferred that the extract may be acting on the adrenergic receptors. It is also possible that some of the constituents stimulated the muscarinic receptors in the uterus. The uterine contractile effects of medicinal plants have been attributed to stimulation of muscarinic receptors in the uterine tissue or through the synthesis and release of prostaglandins well known to be myometrial stimulants reported to mediate the activity of most drugs that stimulate uterine contraction (Solloff, 1979). Although, this work could not establish the latter, the probable involvement of muscarinic receptors in the uterine contractile effect of the extract was supported by the slightly reduced contraction observed when the tissue was pre-treated with atropine before the water extract. Atropine is a well known antagonist of muscarinic receptors. Uterine contractile effects of the leaf extract and stem bark of *Musanga cecropioides,* leaves of *Agapanthus africanus* and *Monechma ciliatum* were earlier reported to be significantly reduced in the presence of atropine (Ayinde et al., 2006; Kamanyi et al., 1992; Veale et al., 1999; Uguru et al., 1998).

The fact that the chloroform fraction attenuated the

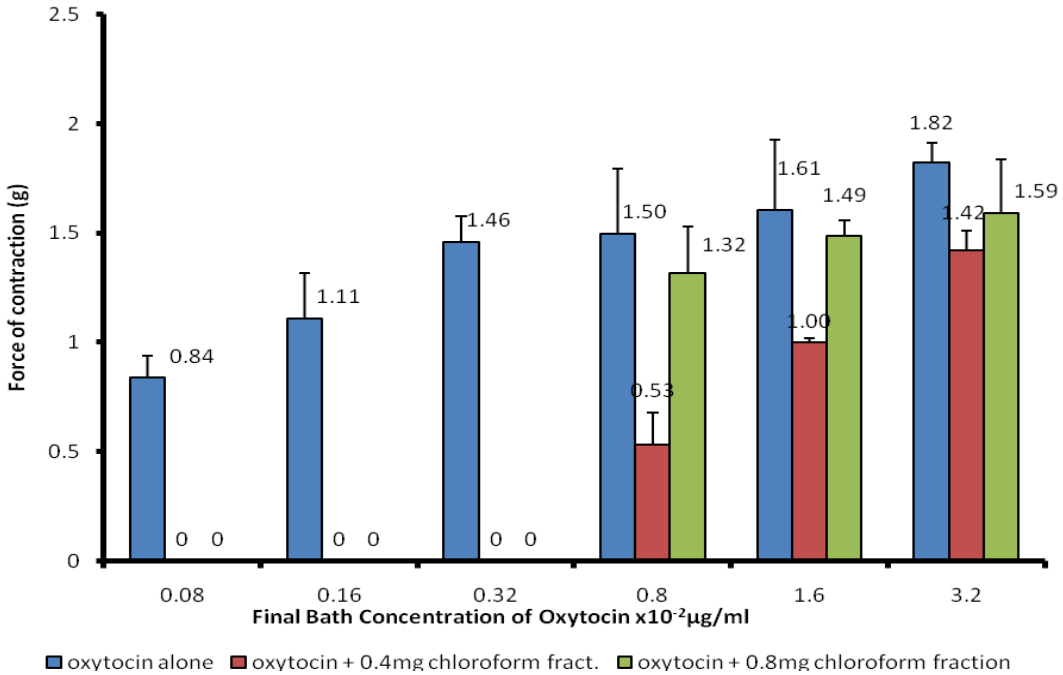

Figure 5. The inhibitory effects of chloroform fraction of *S. sporganophora* on the contractile effects of oxytocin on rat uterus.

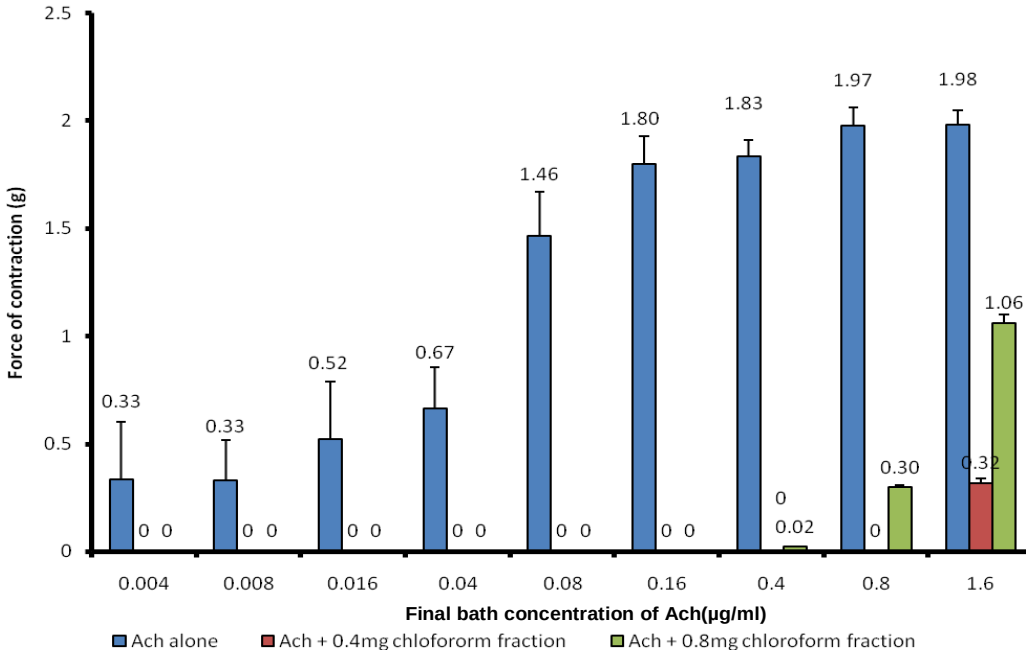

Figure 6. The inhibitory effects of chloroform of *S. sparganophora* on the contractile effect of ach on rat uterine.

contractions occasioned by the administrations of either oxytocin or acetylcholine indicated the probable presence of constituents capable of suppressing both oxytocin and muscarinic receptors in the uterus. Also, it seems that inhibition posed by the chloroform fraction (0.4 and 0.8 mg) was competitive in nature and transient as the

contractions produced by the drugs were later restored albeit at higher concentrations of each drug.

The results of this work have shown that the leaves of *S. sparganophora* have the potential to effect contraction of the uterus and the activity of the plant is not enhanced by partial separation of the constituents.

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