# PHYTOCHEMICAL EVALUATION AND IN VITRO MYOMETRIAL INHIBITION OF THE METHANOL EXTRACT AND PARTITIONED CHLOROFORM FRACTION OF SCOPARIA DULCIS LINN (SCROPHULARIACEAE) ON THE NON-PREGNANT RAT UTERUS.

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## ABSTRACT

Scoparia dulcis (Linn) has been documented as a traditional treatment for the induction of labour and abortion. This study was aimed at investigating the phytochemical composition of the whole plant and the effect on the uterus of the methanol extract (ME) and partitioned chloroform fraction (CF) of the whole plant of Scoparia dulcis, as well as elucidating its possible mechanism of activity. The study revealed that the powdered whole plant of Scoparia dulcis contains the following phytochemicals- indole and isoquinoline alkaloids, saponins, tannins, flavonoid, carbohydrates and reducing sugars. Scoparia dulcis contains many different components as evidenced by the thin layer chromatographic separation. ME (1mg) and CF (1mg) exhibited an inhibitory effect on the concentration-response curves induced by oxytocin (OXY), acetylcholine (ACh) and potassium chloride (KCl) on the rat uterus and significantly reduced ($p < 0.05$ and $p < 0.0001$) the contractile response. A similar effect was observed with salbutamol and atropine respectively on the concentration response curves obtained by OXY, ACh and KCl. The above results suggest the tocolytic effect of ME and CF to be associated with the inhibition of extracellular calcium influx. The results do not justify the use of Scoparia dulcis as a labour-inducer and abortifacient, but rather revealed its possible therapeutic value for the control or management of preterm labour.

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## INTRODUCTION:

The universal role of plants in the treatment of disease is exemplified by their employment in all the major systems of medicine irrespective of the underlying philosophical premise. As examples, we have Western medicine with origins in Mesopotamia and Egypt, the Unani (Islamic) and Ayurvedic (Hindu) systems centred in western Asia and the Indian subcontinent and those of the Orient (China, Japan, Tibet, etc.). How and when such medicinal plants were first used is, in many cases, lost in pre-history, indeed animals, other than man, appear to have their own material medica[1].

The World Health Organization (WHO) defines traditional medicine as: "the sum total of knowledge, skills and practices based on the theories, beliefs and practices indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses" [2]. In some Asian and African countries, 80% of the population depend on traditional medicine for their primary healthcare needs. When adopted by other populations (outside of its indigenous culture), traditional medicine is often called complementary and alternative medicine (CAM)[2].

Scoparia dulcis, an erect, shrubby herb up to 100cm high which usually has many axillary shoots and reproduce from seeds. The stem is more or less woody, ribbed, many branched and glabrous. The leaves are opposite or three at a node, oval or narrowly-oblanceolate, about 2.5-5cm long and 1.5cm wide, widely toothed at the upper half and entire and wedge-shaped at the lower half. The leaf blade is smooth except that the lower surface has some glandular dots. The inflorescence is a slender raceme with 1 or 2 flowers in the upper leaf axil. The flowers are white or bluish and pedicelled. The fruit is a round capsule[3].

Much research has been carried out on Scoparia dulcis to justify its various traditional uses among which are: Scoparia dulcis is used as antioxidant and antiulcer [4,5], as Antihyperglycaemic agent[6], Cytoprotective / Insulin-Secretagogue agent[5,6], antimicrobial and antifungal agent[7], antitumour[8], Antihyperlipidemic[9], Hepatoprotective[10], Anti-Trypanosomal Immunosuppression / Immunological Boosting[11], anti-inflammatory and analgesic agent [12].

Tocolytic agents include Calcium channel blockers, Oxytocin antagonist, beta2 agonists, Magnesium sulphate, prostaglandin synthetase inhibitors, nitric oxide donors and Atosiban13. However, these drugs are sometimes inadequate and have adverse effects like tachycardia, increased cardiac output, pulmonary oedema, hyperglycemia, cardiac depression and inhibition of neuromuscular transmission[14,15,16]. The wide range of tocolytic agents in use is a testimony to the fact that we still do not have an ideal drug available, hence the need for a search of effective and safe alternative drugs for the treatment of preterm labour. There is no report on the pharmacological effect of Scoparia dulcis on the uterus thus, the study was carried out to ascertains the inhibitory actions of the partitioned chloroform fraction and the methanol crude extract of Scoparia dulcis on the uterus of non-pregnant rats and the need to search for a better and save tocolytic agent necessary for the treatment of preterm labour.

## MATERIALS AND METHOD

*Plant collection and identification*
Whole plants of Scoparia dulcis was collected from Egor Local Government Area of Edo State in September, 2010. The plant was identified as Scoparia dulcis Linn by Dr B. Ayinde of the Pharmacognosy department, University of Benin, and a voucher specimen deposited.

*Sample preparation*
Debris was removed from the plants, and cut into smaller pieces then air dried for three weeks, after which they were placed in an oven at 50oC for 2 hours for complete drying. Thereafter, it was pulverized to a powder in an electric mill and the powder was sieved using a sieve of aperture size 1.0mm.

*Extraction and Fractionation of plant material*
400g of the powdered plant was extracted with methanol (2 Litres) by the method of maceration at ambient temperature for 48 hours and then filtered. The filtrate was concentrated to dryness under vacuum at 40oC. The yield of the extract was then determined. The crude extract was then preserved in a refrigerator at 4oC until needed. 12.00g of the methanolic extract of Scoparia dulcis was dissolved in methanol and successively and exhaustively (3 X 30 ml) partitioned into various fractions using n-hexane and chloroform. The various fractions were concentrated to dryness using rotary evaporator, and their respective percentage yields determined.

*Phytochemical screening of Scoparia dulcis*
Phytochemical analysis was carried out on the powdered sample and a standard method was used [1].

Pharmacological screening of the methanol extract and partitioned chloroform fraction of Scoparia dulcis

*Experimental animals*
Adult female non-pregnant Wistar rats weighing 150 -170 g bred in Ibadan, Nigeria were used. The animals were maintained under standard conditions and had free access to standard diet (Grower's Mash from Bendel Feeds and Flour mill, Ewu, Edo State, Nigeria) and water ad libitum.

The animals were allowed to acclimatize for 14 days before being handled according to standard guidelines for use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory animals, 2002).

*Animal preparation for uterine studies*
The animals were primed with Diethyl-stilboesterol (1mg/kg, intraperitoneally) 24 hours prior to the experiment to stimulate oestrous. The rats were sacrificed under anaesthesia with chloroform and the uterus was quickly isolated and placed in previously warmed and aerated physiological salt solution (De Jalons solution) of the following composition in mM: NaCl-154, KCl-5.63, CaCl2.2H20 -0.648, NaHCO3-5.95 and D-glucose -2.77, to simulate the normal physiological environment of the uterus.

The uterus was then cut into sections of about 1cm each, and the cut section was mounted in an organ bath (50ml) which contained De Jalons solution. The lower end of the tissue was attached to a tissue holder using silk suture, and the upper end was attached to an isometric force-displacement transducer connected to a unirecorder. The physiological salt solution in the organ bath was continuously supplied with oxygen using an aerator, and the temperature was maintained at 37oC. The tissue was allowed to equilibrate for 30 minutes and washed with the PSS every 10 minutes17, 18, before carrying out the following experiments:

Inhibition of uterine contractility
Standard contractile agents- oxytocin and acetylcholine were administered successively in graded doses (oxytocin-0.2, 0.4, 0.8, 2, 4 and 8 IU; acetylcholine-20, 40, 80, 200, 400, 800, 2000, 4000, and 8000μg), and the contractile responses observed on a graph sheet (Ugo Basile) connected to an isometric transducer. The crude methanolic extract and chloroform fraction of Scoparia dulcis were then administered successively at a dose of 1mg.

Standard uterine relaxant drugs-Salbutamol (5μg) and Atropine (5μg) were administered to inhibit the uterine contractions of oxytocin and acetylcholine respectively and their dose-response curves compared to that obtained with Scoparia dulcis.

Relaxant effect on k+ - induced contraction
To determine whether the spasmolytic activity was through

calcium channel blockade, K+ was used to depolarize the preparation using a modified method of Farre et al., 19. K+ (80mM) was added to the organ bath, which produced a sustained contraction. At this point, Salbutamol and the extracts were then added to the organ bath cumulatively to obtain concentration-dependent inhibitory responses [20,21].

*Drugs and Chemicals*

Diethylstilboestrol, Potassium Chloride, Acetycholine, Salbutamol and Propranolol were obtained from Sigma (UK), Oxytocin (Laborate Pharmaceuticals, India). These were all prepared fresh on the day of the experiment.

*Statistical Analysis*

All values were expressed as mean ± S.E.M (standard error of mean) and n represents the number of rats from which uterine segments were obtained. The EC50 (concentration needed to produce a 50% maximal response) and Emax (maximum achievable response) were computed for each concentration - response experiment. Comparisons were made using one-way ANOVA with Dunnett Multiple Comparison Test. $p < 0.05$ indicated statistical significance in all cases.

## RESULTS AND DISCUSSION

The low yield of the crude methanol extract obtained (7.01%) is an indication that maceration is not a suitable method of extraction. Rather, soxhlet extraction which uses less quantity of extracting solvent gives a higher yield of extract and is a preferable method of extraction although there is the risk of heat destroying the thermolabile constituents in the plant, for that reason use of heat was reserved until absolutely necessary. The partitioned chloroform, aqueous and hexane fractions gave yields of 8.56%, 60%, and 24.90% respectively. This indicates that the methanol extract mainly contains polar components, followed by non-polar components, and a little amount of semi-polar components.

The phytochemical analysis of the powdered plant of Scoparia dulcis showed the presence of carbohydrates, reducing sugars, saponins, isoquinoline and indole alkaloids, tannins, flavonoids and cardiac glycosides (table 1). However tropane alkaloids and anthraquinones were found absent. Isoquinoline alkaloids have been shown to inhibit acetylcholinesterase and have proved useful in the treatment of Alzheimer's disease [22]. They also inhibit inflammation and possess dose-dependent anti-nociceptive ability[23] and have been shown to inhibit the movement of second-stage larvae of Toxocara canis [24].

| SECONDARY METABOLITES | RESULT |
|---|---|
| Carbohydrates | + |
| Reducing sugars | + |
| Saponins | + |
| Alkaloids | + |
| Tropane alkaloids | - |
| Isoquinoline alkaloids | + |
| Indole alkaloids | + |
| Tannins | + |
| Flavonoids | + |
| Cardiac glycosides | + |
| Anthraquinones | - |

**KEY:**+ Detected - Not detected

Indole alkaloids inhibit smooth muscle high-conductance calcium-activated potassium channels[25] and protect plants against aphids[26]. Saponins have been shown to elicit spontaneous contraction having large amplitude in pregnant uterus[27].

The Rf value (0.48) obtained from the first spot on the chromatogram of the chloroform fraction using the solvent system (chloroform 3: methanol 2) was exactly the same with the Rf of the second spot (0.48) obtained on the chromatogram of the crude methanol extract. This suggests that the spots on both chromatograms contain exactly the same component.

The methanol extracts (ME) and partitioned chloroform fraction (CF) of Scoparia dulcis exhibited an inhibitory effect on the concentration-response curves induced by oxytocin (OXY). OXY binds to specific receptors to increase internal calcium ($Ca^{2+}$) by inhibiting $Ca^{2+}$ extrusion by the suppression of $Ca^{2+}$-ATPase (the Ca pump); by opening calcium channels; and by stimulating inositol -1,4,5-triphosphate (IP3), which releases internally stored $Ca^{2+}$ [28,29]. Kao[28] described a four-fold action of oxytocin on the myometrium. However, oxytocin's net action is to raise intracellular levels of Ca2+ and thereby depolarize the cells or it may facilitate opening of voltage-dependent ion channels during the process of excitation by action potentials (Kao, 1989). ME and CF inhibit OXY-induced myometrial contractions, suggesting possible interaction by ME and CF with one or more underlying mechanisms responsible for the net action of OXY, directly or indirectly.

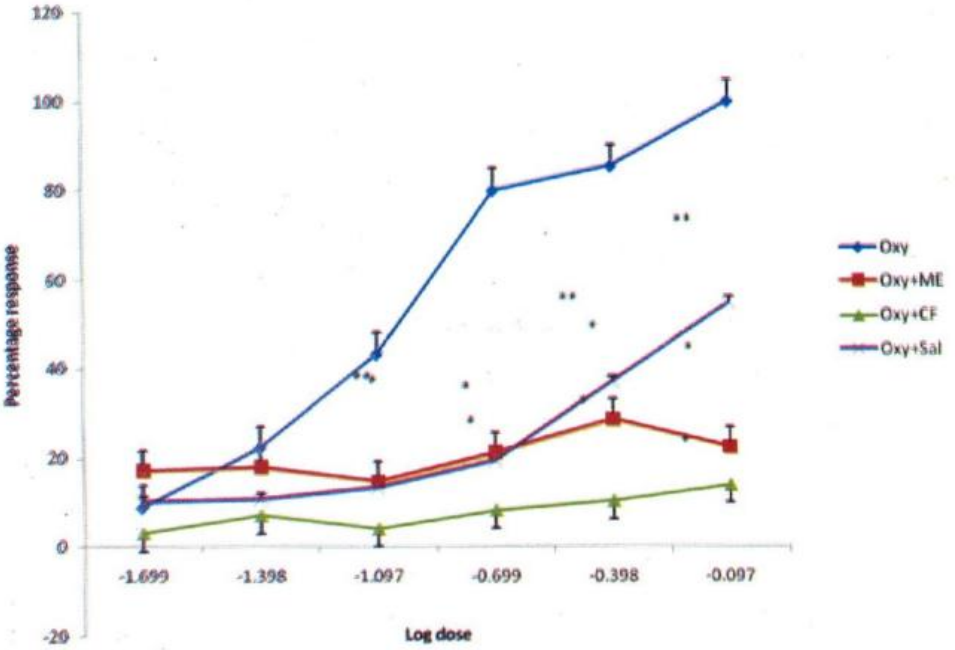


**Fig 1: Effect of the methanol extract and partitioned chloroform fraction of Scoparia dulcis on oxytocin-induced contractions in the non-pregnant rat uterus.**

Values are mean percentage responses ± SEM (n = 6 per group). **P < 0.05, *P < 0.0001 significantly different from oxytocin-induced contractions alone.

KEY:
Oxy Oxytocin-induced contractions alone
Oxy + ME Oxytocin-induced contractions in the presence of the methanol extract
Oxy + CF Oxytocin-induced contractions in the presence of the chloroform fraction
Oxy + Sal Oxytocin-induced contractions in the presence of salbutamol

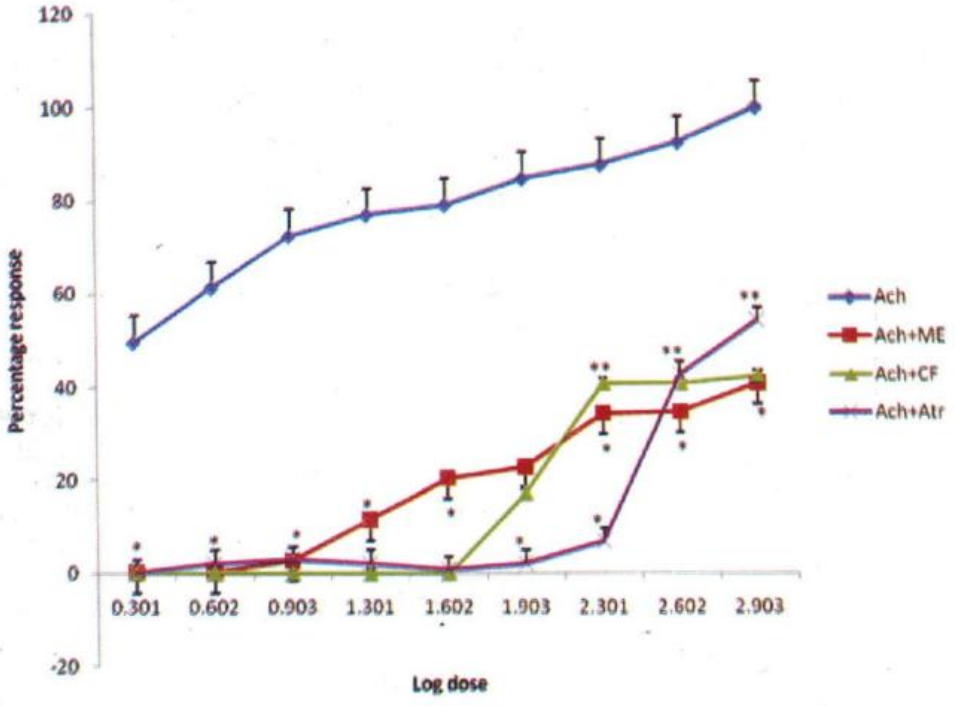


**Fig 2: Effect of the methanol extract and partitioned chloroform fraction of Scoparia dulcis on acetylcholine-induced contractions in the non-pregnant rat uterus**

Values are mean percentage responses ± SEM (n = 9 per group). **P < 0.05, *P < 0.0001 significantly different from acetylcholine-induced contractions alone.

KEY:
Ach Acetylcholine-induced contractions alone
Ach + ME Acetylcholine-induced contractions in the presence of the methanol extract
Ach + CF Acetylcholine-induced contractions in the presence of the chloroform fraction
Ach + Atr Acetylcholine-induced contractions in the presence of Atropine

Salbutamol (SAL), a known myometrial relaxant was employed in this study to compare its effects with those of ME and CF. SAL is a non-selective β-adrenoceptor agonist with greater affinity for the β2-adrenoceptor subtype, and β2-adrenoceptors are responsible for causing smooth muscle relaxation on many organs, the uterine smooth muscle inclusive[36]. CF inhibited OXY-induced contractions to a greater extent than did SAL (Fig 1). Also, Acetylcholine (ACh)-induced contractions were significantly inhibited by ME and CF (Fig 2). ACh induces contraction of smooth muscles via muscarinic receptors. Atropine is a known specific muscarinic receptor antagonist and inhibits the muscarinic receptor-mediated actions of ACh. Significant inhibition of ACh-induced uterine contractions by ME and CF suggests possible anti-muscarinic activity.

Table 2: Percentage inhibition of potassium chloride-induced sustained uterine contractions by the methanol extract of Scoparia dulcis

| DOSE | INHIBITION (%) |
|---|---|
| ME (2mg) | 39.26 ± 14.80 |
| ME (4mg) | 54.63 ± 11.73 |
| ME (8mg) | 72.31 ± 7.06 |
| ME (10mg) | 89.08 ± 3.28 |
| ME (12mg) | 91.02 ± 2.38 |

KEY: ME Methanol extract

Table 3: Percentage inhibition of potassium chloride-induced sustained uterine contractions by the partitioned chloroform fraction of Scoparia dulcis

| DOSE | INHIBITION (%) |
|---|---|
| CF (2mg) | 49.22 ± 3.90 |
| CF (4mg) | 69.10 ± 1.64 |
| CF (8mg) | 94.44 ± 3.21 |

KEY: CF Chloroform fraction

Table 4: Percentage inhibition of potassium chloride- induced sustained uterine contractions by salbutamol.

| DOSE | INHIBITION (%) |
|---|---|
| SAL (2µg) | 84.60 ± 4.94 |
| SAL (5µg) | 89.47 ± 4.72 |
| SAL (10µg) | 92.59 ± 2.76 |
| SAL (15µg) | 94.02 ± 2.49 |
| SAL (20µg) | 95.45 ± 2.23 |
| SAL (25µg) | 96.88 ± 1.97 |

KEY: SAL Salbutamol

Horowitz et al., [31] showed that a solution high in potassium ions (K+) produces depolarization of the membranes resulting in the opening of the L-type voltage-dependent ion channels to cause an influx of $Ca^{2+}$ and eventually muscle contraction. In this study, ME and CF exhibited inhibitory effects on OXY-induced as well as KCl-induced contraction (Figs 1; Tables 2 and 3). Inhibition of sustained contractions induced by potassium chloride was remarkable with both ME and CF, but not comparable to that observed with salbutamol (Table 4). These results suggest possible inhibition of extracellular $Ca^{2+}$ influx[32]. However, further work will be done to confirm the actual mechanism of action, and also to isolate the active principles responsible for the observed myometrial inhibition.

## CONCLUSION

This study revealed that the powdered whole plant of S. dulcis contains the following phytochemicals- indole and isoquinoline alkaloids, saponins, tannins, flavonoid, carbohydrates and reducing sugars. Scoparia dulcis contains many different components as evidenced by the thin layer chromatographic separation. ME and CF significantly inhibited oxytocin- and acetylcholine-induced contractions of the non-pregnant rat uterus at a concentration of 1mg. These findings do not justify the use of Scoparia dulcis as a labour-inducer and abortifacient in Ayurvedic medicine in India, but rather revealed its potential therapeutic value for the control or management of preterm labour.

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