Original Article

Extraction and Demonstration of Uterotonic Activity from the Root of *Steganotaenia Araliacea Hochst*

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ABSTRACT

Background: The root of *Steganotaenia araliacea* is used for assisting labour in folk medicine. Recent reports indicate that the root could possess uterotonic substances.

Objectives: The study aimed to evaluate three methods for the extraction of uterotonic principles from the root of *S. araliacea* growing in Zambia.

Methods: Roots of the plant were collected from Chongwe District of Zambia. The air-dried roots were size-reduced, and the powdered material extracted with hot ethanol, hot distilled water, and cold distilled water. The solvent extracts were concentrated and dried at 110 ^{0}C. Solutions of the hot aqueous and cold aqueous extracts were prepared in distilled water and used for organ bath experiments to demonstrate uterotonic activities using strips of pregnant rat uterus. The frequencies and amplitude of contractile forces were recorded. The amplitudes were plotted against log concentration of extract with GraphPad Prism software, and the EC50 values determined.

Results: The percentage yields were 31.3 % for the hot aqueous extract, 8.15 % for the ethanolic extract, and 3.27 % for the cold aqueous extract. The cold aqueous extract showed higher potency (EC50 of 0.54 mg/ml) compared to the hot aqueous extract (EC50 of 2.09 mg/ml).

Conclusion: Root extracts of *S. araliacea* possess demonstrable uterotonic effects. Extraction of the roots for this purpose could benefit from preliminary defatting with organic solvents, followed by successive extraction with hot and cold water.

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INTRODUCTION

The demand for revitalization of health care is ever on increase, parallel to this is the demand of services of Traditional Medicines. The use of Traditional Medicines all over the world is demonstrating to be phenomenal. Traditional medicine has a great potential of therapeutic benefits in its contribution to modern medicine. Presently more than 30% of marketed modern medicines are derived directly or indirectly from medicinal plants[1].

WHO estimates that 80% of the population in developing countries depends on traditional medicines for their primary health care [2]. The world market for Traditional Medicines has reached US$60 million, with annual growth rates between 5 and 15%. Traditional medicinal plants and products can be targeted for patent claims since they have become of great interest to the globally organized herbal drug and cosmetic industries[3]. Zambia is a member of World Trade Organization since 1995.

Keywords: *Childbirth, extraction methods, medicinal plant, potency, Steganotaenia araliacea, uterotonic effect*

Under Article 27 of Trade Related Intellectual Property Rights Agreement; members will provide for the protection of plant varieties either by patents or by an effective *sui generis* system or by any combination thereof[4]. With the Doha declaration on TRIPS agreement and Public Health, it becomes important for us to protect our Intellectual Property Rights in the form of Indigenous Knowledge Systems (IKS)[5].

IKS generally refers to age-old long-standing traditions and practices involving wisdom, knowledge and teachings of communities and encompassing traditional technologies[6].

For the most part, the knowledge is passed down through generations, usually from designated persons to others using various modes of communication like story-telling, rituals, songs or laws. This framework enables growth and sustainability of communities which invariably includes sustaining the health of community members.

It can, therefore, be said that a portion of what indigenous knowledge imparts is traditional medicine knowledge[7]. These therapeutic practices have been in existence for hundreds of years before the development of conventional scientific medicines e.g. morphine from *Papever somniferum*, quinine from *Cinchona officinalis*, strychnine from *Srychnox nuxvomica* and ephedrine from *Ephedra sinica*[8].

To preserve these knowledge systems, the regional office in Brazzaville, responsible for African region has facilitated activities leading to the present trend of recognition and development of herbal medicines in Africa by various international organisations. This calls for positive response from all the medical and pharmaceutical research scientists of African origin. The decade 2001-10 was tagged as 'Decade of Traditional Medicine in Africa and 31st August is celebrated every year as the, 'Traditional Medicine Day'[8].

Traditional health practitioners (THPs) have used herbal plants and other remedies for beneficial effects during pregnancy, to induce labour, in the removal of retained placenta and management of post-partum bleeding[9]. The effects of these medicines have been reported informally, mostly in circumstances where their effects have been rather excessive causing injury to the uterus (rupture) or death by peri partum heamorrhage. These exaggerated responses are suspected to be related to overdosing since there are no regulated traditional measurements. This study compared uterotonic activities in extracts of *Steganotaenia araliacea Hochst* obtained using three different solvents and conditions. The purpose was to optimise the extraction process for uterotonic principles from the root.

METHODS

A. Plant collection and identification

The investigators consulted with local people in Chongwe District to help locate the plant. Root samples of the plant were collected from the Chongwe District of Zambia using standard procedures. The samples were immediately placed in a black plastic bag to prevent any possible solar degradation of active compounds. For taxonomic verification, specimens of the plant were dried by placing them in a plant press and sun drying for about three days while changing blotting papers each day. The plant was identified to species level in the field using prior knowledge and later confirmed by physical comparison with specimens in the herbarium at University of Zambia (UNZA) and by consulting the taxonomic literature. Voucher specimens were deposited in the UNZA herbarium.

B. Processing and extraction of the roots

Preliminary Treatment

The roots were freed of soil and debris, and size reduced using a clean laboratory axe and then air-dried in the shade for 14 days under blotting paper. Moisture content was monitored to a constant level

of 6 % (wt/wt), as a function of weight loss. A laboratory blender (Warring Commercial, Christison Particle Technologies, UK) was used to reduce the samples further, and the powdered material stored in Ziploc plastic bag.

Extraction

The powdered sample was subjected to three types of extraction; soxhlet, hot and cold water extraction.

Soxhlet extraction using ethanol (distilled)

Approximately, 302 g of the powdered sample was extracted with about 2600 ml of distilled ethanol for about 10 hrs using soxhlet apparatus. This was followed by solvent evaporation using a rotary evaporator (Rotavapor R11, BUCHI, Switzerland) at 45°C. The resultant glue-like residue was further dried in an oven at 80°C for 48 hours. The resulting dry extract was stored in the refrigerator at 4 °C.

Hot water extraction

Fifty grams (50.0 g) of the powdered sample was extracted with 800 ml of boiling distilled water for 10 min. The extract was cooled to room temperature, and then centrifuged at 4000 rpm for 10 min. The supernatant was decanted and evaporated to near dryness using a hot plate, and further dried in an oven at 110 °C. The dry extract was stored in the refrigerator at 4 °C until required for analysis.

Cold water extraction

Approximately, 302.16 g of the powdered sample was soaked in 1,750 ml cold distilled water overnight. The mixture was centrifuged (4000 rpm for 10 min) and decanted to obtain a clear solution. The decanted supernatant was evaporated to near dryness with a hot plate, and then dried in an oven at 110 ° C. The extract was then stored in the refrigerator at 4 °C.

C. Habituation of the animals and isolation of uterine muscle

Pregnant Female Wistar albino rats aged 7 weeks and weighing between 180 to 200 g were obtained from the School of Veterinary Sciences of the University of Zambia, Lusaka. They were housed in standard metal cages, maintained at room temperature (20 -24 ° C), 12 hours light/dark cycles, and allowed free access to water and rodent pellets (obtained from Tiger Animal Feeds Ltd, Lusaka).

D. Demonstration of uterotonic activity on isolated uterine muscle

On the 10th day of gestation, the rats were sacrificed by cervical dislocation, immediately dissected and the uteri extracted. Any adherent fat, connective tissues, and the foetuses were removed. The uteri were placed in De Jalon's physiological solution at room temperature, and longitudinal strips (about 2 cm long) were cut out from the cleaned uteri and mounted in the organ baths (AD Instruments) containing about 25 ml De Jalon's solution and maintained at about 32 °C. The force transducers were connected to PowerLab and LabTutor (AD Instruments). Baseline spontaneous contractions were recorded for 5 minutes. Thereafter, solutions of the cold aqueous extract were added to give final bath concentrations varying from 0.016 mg/ml to 2.048 mg/ml with 3 washes between additions of each concentration. The contractions produced by each concentration were recorded on the Lab Tutor for 5 minutes. Using the same tissue, the above procedure was repeated for the hot aqueous extract. The procedure was not carried out for the alcoholic extract as it failed to dissolve in water or saline. Oxytocin was used as positive control; it also served to demonstrate sustained viability of the isolated uterine tissues during the experiments.

E. Data Analysis

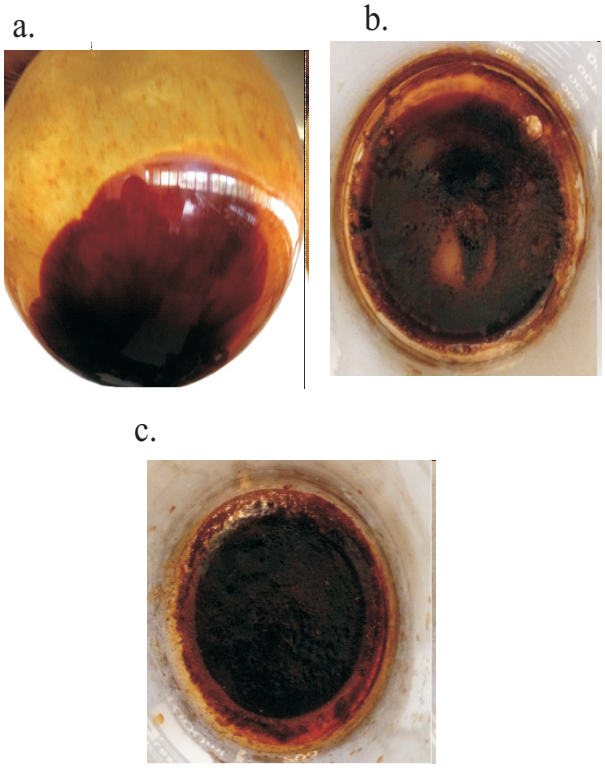
The contractions of the uterine muscle produced by graded doses of the hot and cold crude aqueous extracts were analysed to determine the frequency (number of contractions per minute) and magnitude of the force of contraction (the difference between the peak and baseline). Prism GraphPad software (version 7) was used to plot the response against the logarithm of concentrations of the extract. The EC_{50} values and Hill Slope factors were determined.

RESULTS

Description of the extracts

The alcoholic extract yielded a reddish-brown oily viscous residue. This could not dissolve in water or normal saline and was not analysed further. The cold-water extract gave a brownish-black gummy residue that was readily soluble in water. This was reconstituted with cold distilled water and used for the isolated organ experiments. The hot extract was a semi-solid dark-brown residue that was also soluble in water. After reconstitution with distilled water, it was also used for the organ bath studies.

Figure 1: Physical appearance of: a. Hot-ethanolic extract, b. Hot-water extract, and c. Cold-water extract



The Percentage Yield

Hot water extract of root exhibited highest yield (31.3%) followed by ethanolic Soxhlet extract (8.15%). The cold water extract showed the lowest yield of 3.27%.

Table 1: Percentage yield of crude extracts of root of *Steganotaenia araliaceais Hochst*

	Soxhlet	Hot water	Cold water
Mass of powder (g)	302.0	50.0	302.16
Mass of extract (g)	24.62	15.65	9.87
Yield (%)	8.15	31.30	3.27

Organ Bath Studies

The cold aqueous extract markedly increased the amplitude and frequencies of contractions of the uterine muscles in a concentration dependent manner, with the lowest tested dose, 0.016 mg/ml, producing demonstrable increase in contraction frequency. The hot aqueous extract produced observable increases in the frequencies and amplitudes of contractions of the uterine smooth muscle, but the effects were smaller than the effects of similar concentrations of the cold extract. Oxytocin produced a much higher effect.

Figure 2: Baseline spontaneous rhythmic contractions of the rat uterine smooth muscle

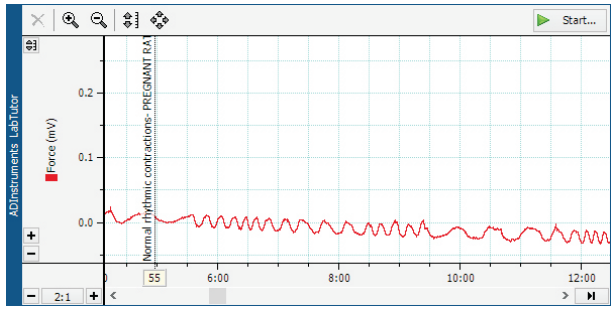
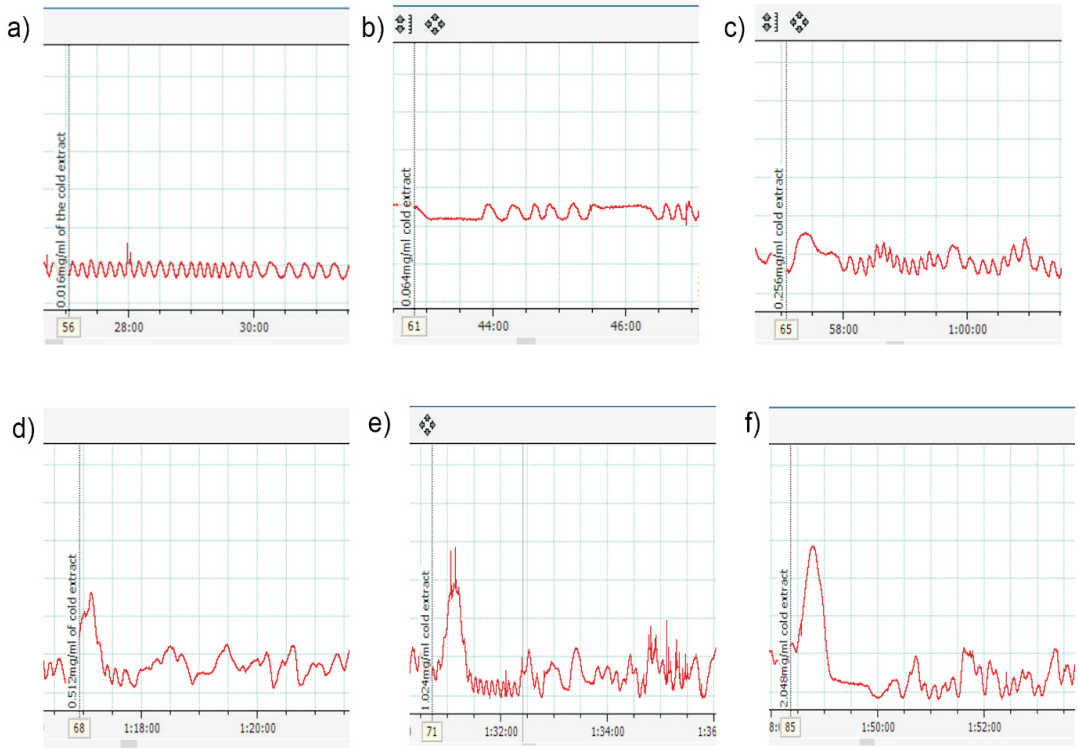
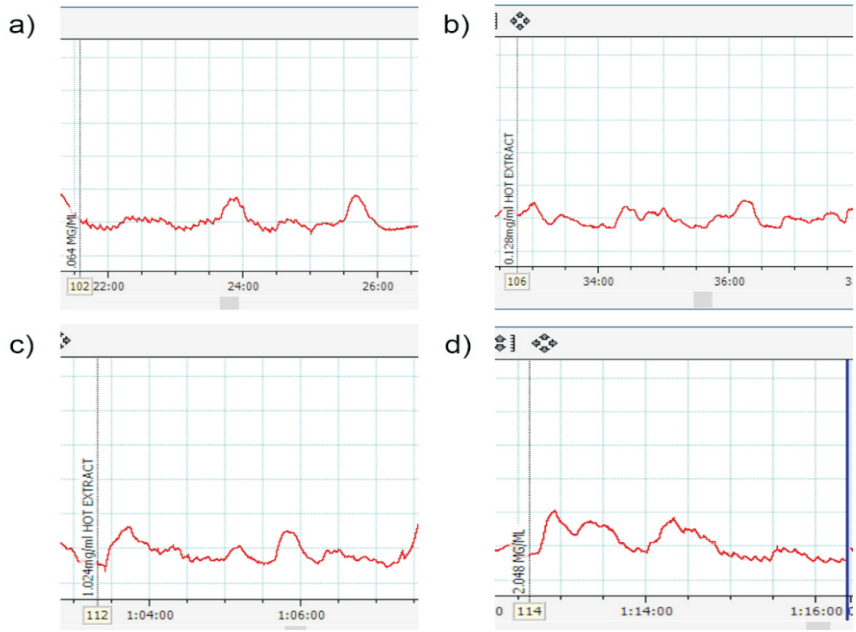


Figure 3: Contractions induced by different concentrations of the cold aqueous extract



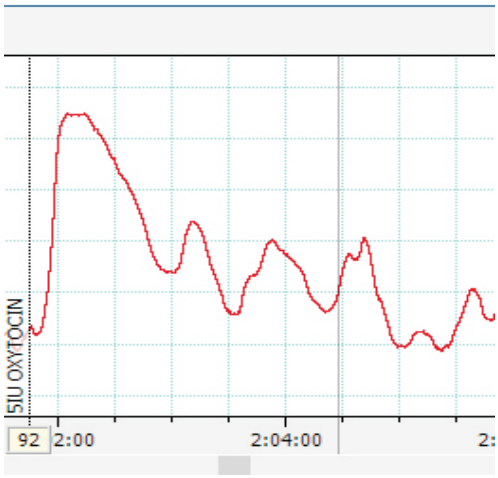
(Final bath concentrations of the cold aqueous extract were a) 0.016 mg/ml, b) 0.064 mg/ml, c) 0.250 mg/ml, d) 0.512 mg/ml, e) 1.024 mg/ml and f) 2.048 mg/ml, respectively).

Figure 4: Contractions induced by different concentrations of the hot aqueous extract



(The final bath concentrations of the hot aqueous extract were a) 0.064 mg/ml, b) 0.128 mg/ml, c) 1.024 mg/ml and d) 2.048 mg/ml, respectively).

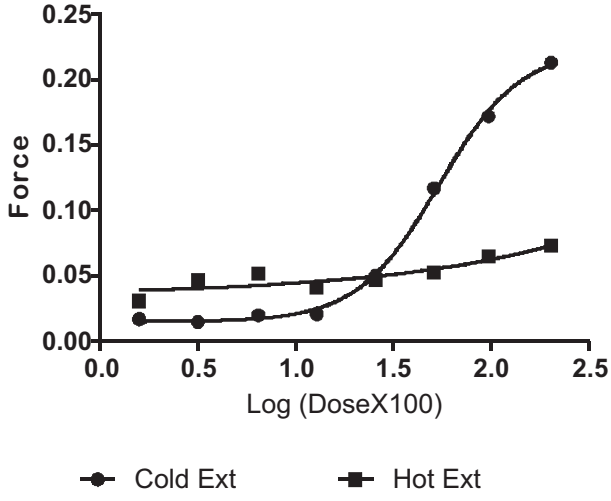
Figure 5: Contractions induced by 5 iu/ml of oxytocin



Concentration (Dose) - Effect Analysis

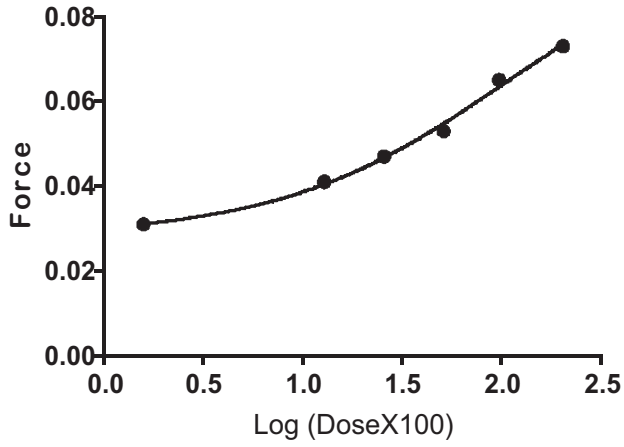
Quantitative analysis of the concentration – response relationships showed that the cold extract was more potent than the hot extract with a lower EC_{50} of 0.58mg/ml compared to EC_{50} of 1.06 mg/ml for the hot extract. The semi-log plot of the cold extract was completely sigmoidal . Plot of the hot extract was remarkably sigmoid; however, outlying points were removed in order to construct a more representative curve from which the $EC_{50}$was calculated.

Figure 6: Concentration (Dose) -response curves for the hot and cold extracts.



(Log (agonist) vs. response -- Variable slope (four parameters), EC50 and Hill Slope factor for cold extract were 0.5428 and 2.09, respectively)

Figure 7: Modified concentration-response curve for the hot extract



(Log (agonist) vs. response -- Variable slope (four parameters) reanalysed omitting outlying points, EC_{50} and Hill Slope factor, for hot extract, were 1.06 and 0.76, respectively)

DISCUSSION

Extraction of *S. araliacea* root with ethanol, hot and cold water resulted in the hot extract having the highest percentage yield of the extract. The ethanolic extract, with a moderate percentage yield (8.15 %) was oily, suggesting that the root contains a reasonable quantity of fatty material. Defatting of the powdered root with organic solvents as a preliminary step in the processing of the root for analysis of its bioactivity is recommended [10], [11]. Furthermore, the oil from the root of this plant could possess properties that may be useful medicinally and/or industrially. Plant oils are widely used in medicine, cosmetology, aromatherapy, biofuel production, and other industrial applications[12, 13, 14, 15, 16]. The study therefore recommends further extraction and distillation of the root oils and investigation of their potential usefulness in these areas.

The hot aqueous extract showed the highest percentage yield, but, uterotonic bioactivity was higher with the cold aqueous extract, which demonstrated a much higher potency in the organ bath studies. This finding probably suggests that hot water extraction removed significant quantities of substances that have no uterotonic activities (artefacts), leaving a purer cold aqueous extract [17]. Based on these observations on the percentage yields and the bioactivities of the extracts, the study further emphasizes that extraction processes for the recovery of uterotonic principles from the root of *S. araliacea* could benefit from preliminary defatting of the powdered material with organic solvents. Hot water extraction to remove artefacts could follow this step, before cold aqueous extraction of the biologically active materials from the root.

The study confirms the presence of uterotonic principles in the root extract. This observation is consistent with previous report[18]. Importantly, it has provided scientific justification for the use of the root by traditional practitioners in maternal healthcare and specifically for assisting labour. Furthermore, the extract could provide a lead for the development of an orally active drug that could serve in obstetrics for assisting women with prolonged labour. Its further development should include purification, identification, and chemical characterization of the active principles. These steps should precede preclinical toxicology, safety pharmacology, and clinical tests in human and/or large animal subjects. The material can also be useful for medical abortion for pregnant women at risk[19,20].

Induction of uterine smooth muscle contraction involves complex interplay of different signalling mechanisms that include neuronal, endocrine, mechanical, and metabolic processes. Current evidence shows that pathways mediated by acetylcholine, oxytocin, histamine, and prostaglandins may be involved, while calcium and other divalent cations and inositol triphosphate play roles as second messengers [21,22]. The exact molecular mechanism by which the constituents of *S. araliacea* interact with these physiological processes to produce uterine contraction is not obvious. Chemical characterization of the extract and in depth pharmacological studies to elucidate these physiological interactions is in process.

In conclusion, extraction of powdered root of *S. araliacea* with ethanol, hot water and cold water yielded crude extracts with significant uterotonic activities. The cold aqueous extract demonstrated higher uterotonic potency than the hot water extract. This article recommends preliminary defatting of the powder with organic solvents to remove the oils, followed by hot-water extraction to remove artefacts that are not uterotonic, before final cold-water extraction of the active ingredients. The product obtained by this process showed great potential for development into a clinically useful drug for obstetric use.

This is work in progress, and much more work is ongoing to isolate and chemically characterise the active principles and possibly translate this product to the bench side.

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DECLARATION OF INTEREST

The authors declare no conflict of interest associated with this work and have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this write-up. This includes employers, consultancies, honoraria, patents (received or pending), expert testimony, stock ownership, or royalties.

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