EVALUATION OF UTEROTONIC ACTIVITY AND PHYTOCHEMICAL PROFILE OF *GREWIA SIMILIS* K. SCHUM

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EVALUATION OF UTEROTONIC ACTIVITY AND PHYTOCHEMICAL PROFILE OF *GREWIA SIMILIS* K. SCHUM

By

Meshack Damian Lugoba

A Dissetation Submitted in (partial) Fulfilment of the Requirements for the Degree of Master of Pharmacy in Pharmacognosy of Muhimbili University of Health and Allied Sciences

October 2021

CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by the Muhimbili University of Health and Allied Sciences a dissertation entitled **"Evaluation of Uterotonic Activity and Phytochemical Profile of *Grewia similis* K. Schum"** in fulfillment of the requirements for the degree of Master of Pharmacy (Pharmacognosy) of Muhimbili University of Health and Allied Sciences.

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Prof. Sheila M. Maregesi

(Supervisor)

Date: _____

_____

Dr. Rogers Mwakalukwa

(Supervisor)

Date: _____

DECLARATION AND COPYRIGHT

I, **Meshack Damian Lugoba**, declare that this **dissertation** is my own original work and that it has not been presented and will not be presented to any other University for a similar or any other degree award.

Signature …… Date ……....….....

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DEDICATION

This work is dedicated to my late father Dr Damian Lugoba and my beloved mother Agnes Sylivanus, who had always wished me success in my life.

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ABBREVIATIONS

ANOVA	Analysis of Variance
AT	Atropine
AVMA	American Veterinay Medical Association
CNS	Central Nervous System
DCM	Dichloromethane
DES	Diethyl Stilbestrol
DMSO	Dimethyl sulphoxide
DP	Diphenhydramine
EEC	European Economic Community
EC_{50}	Effective Concentration needed to produce a 50% maximal response
E_{max}	Maximum achievable response
EtOAc	Ethyl acetate
EtOH	Ethanol
GSBE	*Grewia similis* Stem Bark Extract
GSBE-1	Dichloromethane fraction from *Grewia similis* Stem Bark Extract
GSBE-2	Ethyl acetate fraction from *Grewia similis* Stem Bark Extract
GSBE-3	Ethanol fraction from *Grewia similis* Stem Bark Extract
GSLE	*Grewia similis* Leaf Extract
ICCUPP	Institutional Animal Care and Use Policy and Procedures
IRB	Institutional Review Board
ITM	Institute of Traditional Medicine
MUHAS	Muhimbili University of Health and Allied Sciences
MX	Meloxicam
NC	Negative Control
NF	Nifedipine
OT	Oxytocin
PC	Positive Control
PSS	Physiological Salt Solution
ROCs	Receptor Operated Calcium channels
RT	Room Temperature

SD	Standard Deviation
THPs	Traditional Health Practitioners
TLC	Thin Layer Chromatography
UDSM	University of Dar es Salaam
VLC	Vacuum Liquid Chromatography
VOCs	Voltage Operated Calcium channels
WHO	World Health Organization

DEFINITIONS OF TERMS

Obstetric and gynecological cases- refers to issues occurring in the care of women during pregnancy and childbirth and in the diagnosis and treatment of diseases of the female reproductive organs (Tripathi, 2008).

Phytoconstituents/Phytochemical classes - are the groups of chemical compounds occurring naturally in the plants that may or may not give the medicinal action of the plant, also known as secondary metabolites (Evans, 2009)**.**

Uterotonic- an agent used to induce contraction, stimulation or increase tonicity of the uterus (Tripathi, 2008).

Uterine receptors- are receptors that mediate the contractile or relaxed responses of the uterus upon stimulation or inhibition by intrinsic or extrinsic factors (Tripathi, 2008).

ABSTRACT

Introduction: *Grewia similis* belongs to the family Tiliaceae, its stem bark and leaves are traditionally used by traditional healers in Tanga Region for various conditions including; induction of labour in pregnant women and as an abortifacient.

Aim of the study: The purpose of this study was to evaluate uterotonic activity, phytochemical classes and compound(s), and possible mechanisms by which extracts of *G. similis* stem bark and leaves stimulate uterine contraction.

Methodology: The study was experimental design conducted from April to July 2021. The plant materials were collected from Tanga Region in August 2020 and the plant authentication was done at University of Dar es Salaam (UDSM). Extracts were prepared by exhaustive cold maceration using 95% ethanol, dried in a rotary evaporator and then fractionated by Vacuum Liquid Chromatography (VLC) using dichloromethane (DCM), ethyl acetate (EtOAc), and ethanol (EtOH).

Ex vivo uterotonic activity was carried out using healthy female young adult rats weighing between 120 and 200 g. To deduce the possible mechanism(s) of action, different concentrations of plant extracts were administered alone and in the presence of the relevant uterine receptor antagonists. Phytochemical analysis was done by using standard qualitative procedures of colour reactions.

Results of all quantitative data were expressed as mean ± SD (n=3), EC_{50} and E_{max} were determined, analyzed by one-way ANOVA followed by post hoc Dunnett's Multiple Comparison test, p <0.05 was considered as a significant level. For phytochemical evaluation, qualitative results were reported as positive or negative.

Results: The extraction yields were 2.5 and 1.6% for barks and leaves, respectively, while the fraction yields were 48.5, 39.3 and 7.9% for DCM, EtOAc and EtOH, respectively. Crude ethanolic bark and leaf extracts of *G. similis* and fractions from the stem bark were shown to cause rat uterine muscle contraction. The crude stem bark ethanolic extract (GSBE) exhibited high uterotonic activity as compared to crude leaf ethanolic extract (GSLE), significant differences (p < 0.05) were observed in the EC_{50} and E_{max}. EtOH fraction from the stem bark of *G. similis* (GSBE-3) had shown high efficacy in uterotonic activity, statistically significant difference in the EC_{50} and E_{max}, when compared to GSBE, stem bark EtOAc fraction (GSBE-2) and stem bark DCM fraction (GSBE-1) (p < 0.05). In deduction of the possible mechanism of action through uterine contraction inhibition, significant differences (p < 0.05) were observed in the EC_{50} and E_{max} of

GSBE-3 alone and in the presence of some of uterine receptor antagonists. Pre-treating the isolated uterine tissue with either calcium channel blocker-nifedipine, prostaglandins receptor antagonist-meloxicam or histamine receptor antagonist-diphenhydramine before administering the extract showed an inhibitory effect on uterine contraction while in the presence of muscarinic receptor antagonist-atropine no effect was observed. The phytochemical screening revealed the presence of tannins, flavonoids, steroids and saponins.

Conclusion: This study had revealed the uterotonic activity of crude ethanolic extracts and fractions of *G. similis* stem barks. Both polar and non-polar fractions of the stem bark extracts exhibited uterotonic properties (most polar fraction i.e, GSBE-3, being the most active). The pharmacological effects/possible mechanisms results suggest that the stimulation of uterine contractility by the stem bark ethanolic fraction of *G. similis* may arise from the interference with calcium channels, stimulation of prostaglandin synthesis and/or activation of histamine H_1-receptors in utero. Uterotonic activity obtained could be due to the phytochemicals detected in *G. similis*.

Further work is needed to isolate and identify the active metabolites and their mechanisms of uterotonic action, toxicological studies, and standardization/formulation of *G. similis* bark and leaf products.

Keywords: *G. similis*, uterotonic activity, uterine receptors, phytochemicals**.**

CHAPTER ONE

1. INTRODUCTION

1.1. BACKGROUND

Traditional medicine has a long history of serving people all over the world. According to the World Health Organization (WHO), about 65-80% of the world's population in developing countries depends essentially on plants for their primary health care, including gynecological and obstetric related issues, due to poverty and lack of access to modern medicine (WHO, 2013).

Herbal medicine usage in Africa, especially in rural areas gradually increases day-to-day. Women and children, who form the largest proportion of people, rely mainly on herbal medicines because their health care needs are higher (Kamatenesi-Mugisha and Oryem-Origa, 2007). Furthermore, herbal medicines are extensively used for gynecological and obstetric disorders in many parts of Africa including Tanzania, especially in rural areas (Flandermeyer *et al.*, 2010).

In Sub-Saharan Africa, studies on the estimated prevalence of local herbal use during pregnancy range between 40 and 90% (Liwa *et al*., 2014; Mbura *et al*., 1985),where majority user are rural women which prefer herbal medicines rather than modern medicines for labour induction, menstrual irregularity, conception disorders, sterility, delivery problem, and many such gynecological disorders, because modern medicinal facilities are scanty, which makes them inaccessible to many rural people (Gruber and O'Brien, 2011). Moreover, herbal medicines are cheap, easily accessible, have less or no side effects with high efficacy. However, this valuable knowledge has not been properly documented. Nonetheless, traditional knowledge is vanishing gradually as a result of modernization. Therefore, appropriate and accurate documentation of such traditional knowledge and practies can be an excellent contribution to drug discovery (Kisangau *et al.,* 2007; Amri and Kisangau, 2012; Kankara *et al.,* 2015).

In Tanzania, between 60 and 70% of the population seeks health care through the use of traditional medicine for various conditions including induction of labour (Mahonge *et al*., 2006). The prevalence of local herb use in Tanzania for induction of labour in pregnant women ranging between 10.9 and 40% (Fukunaga *et al*., 2020; Dika *et al*., 2017; Rasch and Kipingili, 2009).

Various plants have been documented for solving several gynecological problems including those related to pregnancy and delivery such as, to induce labour, removal of retained placenta and

management of post-partum hemorrhage (Kamatenesi-Mugisha and Oryem-Origa, 2007; Ahmed *et al.*, 2012). Bioactive compounds acting on the uterus are called uterotonic agents and their actions may involve the modulation of uterine contractions at labour, resulting in either stimulation ("uterotonic") or inhibition ("tocolytic") of myometrial muscle contraction (Gruber and O'Brien, 2011). Plants that produce uterine contractions have a similar action to that of oxytocin which stimulates the uterus, causing strong contractions, and thus producing labour, they have abortifacient properties when used during the first months of pregnancy (Kamatenesi-Mugisha and Oryem-Origa, 2007).

In modern medicine labour induction may be necessary in certain conditions such as post-term pregnancy, lack of progress, oligohydramnions, suspected intrauterine growth restriction, preeclampsia/eclampsia, pre-labour rupture of membranes at term and foetal macrosomia (Mozurkewich *et al.*, 2009). However, the currently used interventional therapies for the induction of uterine contractions lack potency and selectivity and sometimes can have harmful side effects to the mother and baby (Gruber and O'Brien, 2011). With regard to the use of herbal medicines for the same purpose users are at health risks since most of them lack important data including, proof of efficacy, dosage, safety evaluation, bioactive phytochemicals as well as mechanism of action(s).

Several medicinal plants that are used to induce labour have demonstrated uterotonic/oxytocic properties in animal experimental studies. The following are just few examples of well-documented ones; *Monechma ciliatum* leaves (Uguru *et al*., 1998), *Musanga cecropiodes* stem bark (Ayinde *et al*., 2006), *Ficus exasperata* leaves (Bafor *et al*., 2010), *Ficus asperifolia* fruits (Watcho *et al*., 2011), *Ficus deltoidea* leaves (Amiera *et al*., 2014), *Nymphaea alba* rhizomes (Bose *et al*., 2014), *Calotropis procera* leaves (Shamaki *et al*., 2015) and *Launea taraxacifolia* whole plant (Iyabo and Hauwa, 2019).

In Tanzania, uterotonic plants have been reported, however most of them are still lacking scientific evidence. Plants like *Bidens pilosa, Commelina africana, Manihot esculenta, Ocimum suave, Sphaerogyne latifolia, Obetia radula,* and *Rubia cordifolia,* just to mention a few, are among the plants reported to possess uterotonic effect (Rasch and Kipingili, 2009).

This study aimed to evaluate uterotonic activity, the phytochemical profile, and possible mechanisms of action (uterine receptors determination) of extracts of the stem bark and leaves of *G. similis* that are used in North-western of Tanzania for induction of labour and claimed to cause abortion when taken during earlier pregnancy.

1.2. PROBLEM STATEMENT

In Tanzania the use herbal medicines during pregnancy exists. A study conducted in Mwanza region reported the prevalence of 23% among delivering mothers at Bugando hospital who had used herbal medicine for the purpose of reduction of labour duration (Dika *et al*., 2017). Whereas, a prevalence of 10.9% was obtained in a study done Kigoma region where pregnant womed used herbal medicines for labour induction purposes (Fukunaga et al., 2020). Unlike in modern medicine practice where uterotonic medicines are administered with special precautions, Traditional Health Practitioners (THPs) are ignorant of the possible side effects that may be caused by herbal medicines prescribed to pregnant women (Mozurkewich *et al.*, 2009).

Herbal medicines with uterotonic effect may cause negative medical problems such as unsafe abortion, uterine rupture due to tetanic contraction, haemorrhage, bowel infarction, infertility, malformation for the newly born babies, and death to both mother and unborn baby (Rasch and Kipingili, 2009). However, herbal medicines with scientific proof of uterotonic potential could be used to develop safe standardized herbal medicines and modern medicines. Despite the use of *Grewia similis* for induction of labour, not much scientific work has been done on this plant. This particular study is therefore aimed to determine uterotonic activity, deduce the possible mechanisms of uterine contractility as well as determining phytochemical contents of *Grewia similis* stem bark and leaves.

1.3. CONCEPTUAL FRAMEWORK

Tanzania is one of the countries rich in diversity of plant species, with a number of plant species being used in traditional medicine to manage diseases in different communities (Maregesi *et al.*, 2007). Ethnomedical studies have revealed a number of medicinal plants that are used by women to manage obstetric and gynecological related issues such as induction of labour (Rasch and Kipingili, 2009). These studies could play a role in the development of standardized herbal medicines or development of safe, efficacious and affordable uterotonic agents, by evaluating uterotonic activity and phytochemicals through laboratory experiments. *Grewia similis* is among those plants claimed to be used by traditional health practitioners in management of obstetric and gynecological problems including induction of labour in Lushoto District, Tanga Region, Tanzania (**undocumented**). Therefore, there is a need to evaluate uterotonic activity and phytochemical profile of this plant as shown in **Figure 1.** in order to develop/standardize uterotonic medicines for human use.

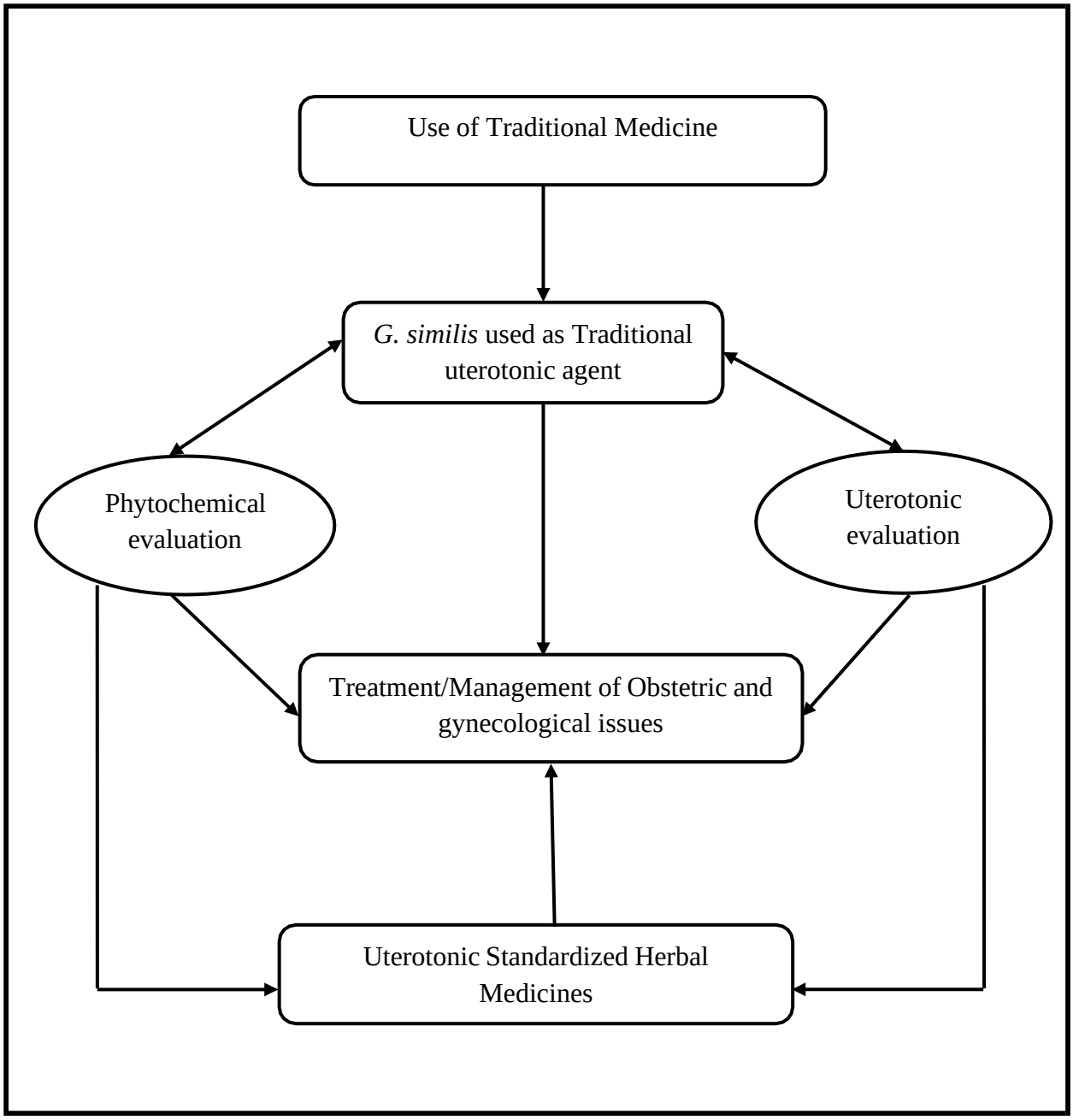


Figure 1: Conceptual framework

1.4. RATIONALE OF THE STUDY

The study provides scientific data on uterotonic activity, uterine receptors involved in the activity, phytochemical groups and active fraction(s) for further work on isolation of uterotonic compound(s) required for drug discovery and development, and/or preparation of a safe standardized uterotonic herbal medicine.

1.5. RESEARCH QUESTIONS

The study was based on the following questions;

i. How does the rat uterus respond when treated with ethanolic extracts of *G. similis* stem bark and leaves?

ii. Which uterine receptors are involved in the uterotonic activity of *G. similis*?

iii. What are the phytochemicals of stem bark and leaves of *G. similis*?

1.6. BROAD OBJECTIVE

The main objective of the study was to evaluate uterotonic activity, phytochemical profile and possible underlaying mechanisms of action by which ethanolic extracts of the stem bark and leaves of *G. similis* stimulate uterine contraction.

1.7. SPECIFIC OBJECTIVES

The specific objectives were:

i. To determine uterotonic activity of crude ethanolic extracts of the stem bark and leaves of *G. similis.*

ii. To identify uterine receptors where the uterotonic of *G. similis* ethanolic extracts act.

iii. To determine phytochemical classes of crude ethanolic extract of the stem bark and leaves of *G. similis*.

1.8. LITERATURE REVIEW

In Tanzania, traditional birth attendants have knowledge of plant materials which can be used to induce labour, and although they might not prepare or administer these preparations, they may instruct women on how to prepare a remedy. It is estimated that 40% of women in rural areas of Tanzania have used plants to induce labour (Rasch and Kipingili, 2009).

Many studies had reported on the traditional uses of the genus *Grewia*, but few of them had reported on the medicinal uses of *G. similis* where by uterotonic activity, phytochemical groups, and mechanism of action of the ethanolic extracts of stem bark and leaves of this plant have not yet been reported.

Uterotonic activity and mode of action

A study conducted in South Africa revealed that, *Grewia occidentalis* L. possesses uterotonic activity and is widely used for induction of labour to facilitate child birth. The most likely mode of action of the remedies is to increase the uterine contractive activity, leading to expulsion of the foetus. (Mulholland *et al.*, 2002; Muithya, 2010).

Contraction of the uterine tissue can be mediated via a number of different receptors such as oxytocin, muscarine, α_1-adrenergic, endothelin-1, prostaglandins-$E_2/F_2\alpha$, histamine-1 and serotonin-2A receptors (Kobayashi *et al*., 1999; Abdalla *et al*., 2004; Hay *et al*., 2010; Gruber and O'Brien, 2011). A study conducted in Nigeria reported that, contractile effect of *Ficus exasperata* was mediated via histamine H_1-and/or α_1-adrenergic receptors, interference with calcium channels and/or stimulation of prostaglandins synthesis in the uterus (Bafor *et al.*, 2010).

The genus Grewia

Grewia species (Tiliaceae) is a genus of flowering plants comprised of 29 species distributed along tropical and temperate regions. The species in the genus *Grewia* have been reported to exhibit a wide range of biological activities and medicinal uses. For instance, *Grewia occidentalis* L. is widely used for medicinal and magical purposes, including induction of labour to facilitate child birth or abortion in South Africa (Mulholland *et al.*, 2002; Muithya, 2010). While other *Grewia* species are reported to be used in the treatment of diarrhoea, gonorrhoea, urinary troubles, and irritation of the bladder, cancer, hookworms, CNS disorders, snakebites, stomach pains, fever,

dysentery, and syphilis. In India, the genus *Grewia* is used to cure urinary tract infections, pneumonia and bronchitis (Jaspers, 1986).

Grewia similis K. Schum

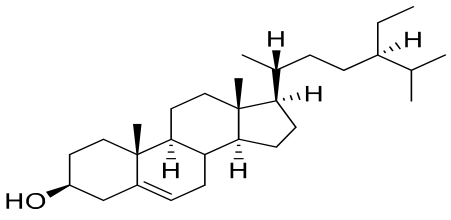
Grewia similis is a straggling shrub or small tree up to 3 m, sometimes a climbing liane with woody knobs on the old stems. The plant is of dry, ever green mountain forest and forest edges, riverine thicket, evergreen bushland or bushed grassland, coastal thicket, 100-300 m. It is widely distributed between 2000-7000 feet above sea level (Muithya, 2010). In Tanzania the plant grows in various regions including Tanga (mostly in Lushoto District), Kagera, Arusha, Morogoro, and Iringa. It is also found in Kenya, Uganda, Sudan, Rwanda and Burundi.

Reported Medicinal Uses

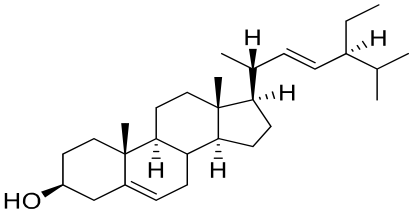
The bark is used to treat wounds, sores and snakebite by the Nyamwezi tribe (Ruffo et al.,2002), and to treat microbial related ailments by the Kipsigis and Maasai tribes (Muithya, 2010). The bark has also found non-human medicinal uses, for example, it is used to treat bicarbonate-intoxicated livestock (cattle). Morever, it is reported to be used for treatment of dental ailments (Maundu *et al.*, 2001; Bussmann *et al.*, 2006), in the management of sexual impotence and erectile dysfunction in western Uganda (Kamatenesi-Mugisha and Oryem-Origa, 2007). The ripe fruits are sweet and eaten raw as snack. The extract of ripe fruits soaked in warm water is used as a sweetener (Ruffo *et al.*, 2002).

Phytochemistry of *Grewia similis*

A study conducted in Kenya reported that, the hexane/dichloromethane (DCM) root extract of *G. similis* showed the presence of steroids and flavonoids. Phytochemical characterization of the isolated compounds from the hexane/DCM root extract of *G. similis* led to identification of 3β-sitosterol and 3β-stigmasterol (Muithya, 2010).



3β-sitosterol



3β-stigmasterol

CHAPTER TWO

2. METHODOLOGY

2.1. STUDY DESIGN AND SITE

This was an experimental study divided in three parts namely; (i) Preparation of crude extract and fractions (ii) Evaluation of uterotonic activity using rat uterus and, (iii) Determination of phytochemical classes.

Experiments were conducted at MUHAS from April to July 2021. The extraction of plant materials and fractionation and determination of phytochemical classes were carried out in the laboratory of Pharmacognosy Department of the School of Pharmacy, and the uterotonic activity testing was performed in the Clinical Pharmacology laboratory of the Department of Clinical Pharmacology at the School of Medicine.

2.2. STUDY POPULATION AND SAMPLE SELECTION

Healthy young adult female white albino rats, aged 8-12 weeks, weighing 120-200 g, were used as study population, where three rats among the population meets the criteria, hence were involved in the study.

2.3. MATERIALS

Albino rats, animal cages (Animal house - MUHAS), Digital weighing scale (Escali, USA), standard animal feed pellets (Hill Pack Ltd, Dar es salaam, Tanzania), 1 ml syringes, gloves, masks, aluminium foils, and cotton wool (Local Pharmacies, Dar es salaam, Tanzania) were used for various purposes in the study.

2.4. CHEMICALS AND REAGENTS

Analytical grade solvents were used for extraction and VLC fractionation. Both solvents and chemical reagents were procured from local community pharmacies and local authorized suppliers (Lab Equip Ltd & Scan Tanzania Ltd, Dar es Salaam, Tanzania). These includes; Methylated spirits, 95% Ethanol, Iron (III) Chloride, Dichloromethane, Ethyl Acetate, Dimethyl sulphoxide, Glucose, Calcium Chloride, Sodium Chloride, Sodium Hydrogen Carbonate, Potassium Chloride. Oxytocin (Steril-Gene Life Sciences Ltd, India), DES (Sigma, UK), Atropine (Health care PVT Ltd-India), Meloxicam (Unichem Lab Ltd-India) and Nifedipine (Cadila Health care Ltd-India).

2.5. PLANT COLLECTION AND IDENTIFICATION

Grewia similis stem bark and leaves were collected from Shume Magamba Forest, Lushoto District in Tanga Region in August 2020. The plant identification using a hebarium specimen was done by a senior Botanist from the Botany Department of the University of Dar es Salaam (UDSM), Mr. Selemani Haji. Voucher specimens with voucher number MLHS5676 were prepared and deposited at the Botany Department, UDSM and ITM herbarium, MUHAS.

2.6. EXTRACT PREPARATION

The plant materials (stem bark and leaves) were dried in an aerated room (under shade), followed by grinding into fine powder by using laboratory electric mill. Local people administer herbal remedies from *G. similis* in aqueous (water) form. However, ethanol may be used as a solvent in extraction because it is safe even when consumed orally by humans at moderate concentrations and evaporate easily during drying. Furthermore, in terms of polarity, ethanol between 80% and 95% is the best safe substitute because it extracts most of the compounds that would be extracted with water and some other organic solvents. Therefore, powdered plant materials (0.5 kg each) were soaked and exhaustively extracted with 2000 ml of 95% ethanol by maceration for a period of 6 days with occasional shaking. Extracting solvent was changed after every 3 days. For the second extraction 3 kg of the powdered stem bark were soaked and extracted with 95% ethanol by maceration. The flasks used for extraction were covered with aluminium foil to prevent evaporation of the solvent and contamination. The extracts were dried using a rotary evaporator (Büchi Labortechnik, Flawil, Switzerland) at 40°C, followed by freeze drying at very low temperature and pressure (Edwards High Vacuum International Crawley, Sussex, England) to remove any traces of solvent.

2.7. FRACTIONATION

Crude stem bark ethanolic extract (26 g) was dissolved in ethanol and adsorbed on 52 g of silica gel (Silica gel 60-Merck KGaA, 64271 Darmstadt Germany) which was later used for loading the sample on the column and subjected to fractionation by VLC, using silica gel as a stationary phase, and eluted sequentially, based on increasing polarity with DCM, EtOAc and EtOH to obtain three fractions. TLC spotting was used to monitor exhaustive elution with each solvent.

2.8. YIELD CALCULATIONS

The percentage yield for the crude ethanolic extract was calculated using this formula:

$$\%\ yield\ of\ crude\ extract = \frac{mass\ of\ the\ crude\ extract\ (g)}{mass\ of\ the\ plant\ material\ (g)} \times 100$$

For calculation of fractions percentage yield, the formula used was;

$$\%\ yield\ of\ fraction = \frac{mass\ of\ the\ fraction\ (g)}{mass\ of\ the\ crude\ extract\ (g)} \times 100$$

2.9. UTEROTONIC ACTIVITY TESTING

2.9.1. Selection of animals for uterotonic activity testing

Experiments were performed using healthy young adult female albino rats, aged 8-12 weeks, and weighing 120-200 g, which were obtained from a well-ventilated room in the laboratory animal center of ITM at MUHAS. The animals were housed in metal cages; allowed to feed food and water *ad libitum* and lighting was controlled to supply 12 h of light and 12 h of dark for each 24 h period. All rats were acclimatized for 4-7 days prior to the oestrus induction.

2.9.2. Preparation of the physiological salt solution (de Jalon's solution)

de Jalon's solution (5 litres) was prepared, which was composed of Sodium chloride (NaCl, 45 g), Sodium bicarbonate ($NaHCO_3$ 2.5 g), Glucose (2.5 g), 21 ml of 10% Potassium chloride (KCl), and 1.35 ml Molar Calcium chloride ($CaCl_2$). de Jalon's solution was selected as it contain low concentrations of calcium ion and the tissue survives long.

2.9.3. The rat uterus and drug preparation

For this experiment the sensitivity of the rat uterus depends upon its conditions. The rats used were brought into estrous by injecting subcutaneously, 48 h beforehand, a dose of DES, 2.0 mg/kg which dissolved in 30% ethanol (de Jalon's *et al*., 1945). After 48 h, animals were anaesthetised and killed humanely by using Intraperitoneal injection of 100-150 mg/kg Sodium pentobarbital ie. 20 mg in 0.3 ml (American Veterinary Medical Association, (AVMA), 2020) and the abdomen opened. The two horns of the uterus were dissected out and transferred to a Petri dish containing

de Jalon's solution at 32-37 ºC. The two horns were separated and freed from fat, and each was cut open longitudinally, so the preparation was a sheet of muscle, instead of a narrow tube. One preparation was taken, a thread was attached at each end and the preparation mounted in the de Jalon's solution aerated with (95% O_2 and 5% CO_2) in a 20 ml organ bath at 32–37 ºC. One thread was attached to a fixed pin and the other to a 500 mg lever fitted with frontal-writing point. The preparation was allowed to equilibrate/normalize for 30 min before adding the test drugs and standard drug/positive control (oxytocin at doses 0.04-0.32 µg).

Exactly 800mg of each tested sample (crude ethanolic bark & leaves and fractions) were weighed using a weighing machine of up to 4 decimal places. The 800 mg of each ethanolic extract were dissolved in 8 ml of 1% DMSO in de jalon's solution. Serial dilutions were further made from the stock solution. To draw the specified amount of the crude drug to introduce to the organ bath, 1 ml new disposable syringes were used. The tissues were mounted in the 20 ml of bathing solution therefore, the actual concentration of the drug extract was that particular concentration drawn using syringe plus the organ bath dilution effect. Thus 100mg/ml was divided by the 20 ml of the organ bathing solution to give the final concentration of 5 mg/ml.

2.9.4. Drug challenges

After 30 min of equilibration period, uterine contractile responses were elicited by adding non-cumulatively oxytocin (OT) as positive control at doses of 0.04-0.32 µg, 1% DMSO in de Jalon's solution as negative control (NC) and crude ethanolic extracts and fractions at doses of 0.3125-20 mg/ml to the PSS. In each consecutive experiment, new de Jalon's solution and crude ethanolic extract and fractions were freshly made. Several cycles were observed on kymograph and preparation was washed after each cycle of the test. Usually, a maximum effect is produced within 30 seconds, so the following cycle was used; 0 min, the kymograph was started, 1 min, the drug was added, and after 1.5 min kymograph was stopped and the preparation was washed. The tissue was always washed with de Jalon's solution every time injected drug (or set drugs) recording was made before another drug (or set drugs) was introduced. The time of tissue washing varied based on the behaviour of the drug on the tissue. The washed tissue in the organ bath was left to normalize/equilibrate before addition of another drug.

Each dose of the drugs was allowed to act for 30 seconds and the effect produced (change in length/amplitude) were measured using a simple lever. The writing point on the tip of the lever

makes mark/curves on the smoked paper attached to the drum of a kymograph by a transparent adhesive tape.

2.9.5. Uterine receptors determination

In order to deduce the possible mechanism(s) of action, stem bark EtOH fraction (0.3125 to 20 mg/ml) was administered alone and in the presence of each of the following antagonists; i. Atropine, a muscarinic receptor blocker (20.00 and 100.00 nM); ii. Nifedipine, a calcium channel antagonist (2.50 and 20.50 nM); iii. Meloxicam, a prostaglandin synthesis inhibitor (1.45 and 14.25 nM); and iv. Diphenhydramine, an histamine H_1-receptor blocker (4.45 and 44.50 nM). In brief, interaction of each of these was separately analysed by introducing the correct calculated dose to the organ bath and left for 5min before subjecting the tissue to the extract, and the contact time was 60 s. The drugs were dissolved in physiological salt solution (PSS). The effect produced (change in length/amplitude) of the fraction alone and in the presence of antagonists were measured using a simple lever on the smoked paper attached to the drum of a kymograph.

2.10. PHYTOCHEMICAL SCREENING

Various chemical tests were performed on the crude ethanolic extract to identify the phytoconstituents using the standard procedures (Harborne, 1998; Evans, 2009; Njoku and Obi, 2009) with slight modifications. Each phytochemical test involved the testing concentrations of 10mg/ml of the crude ethanolic extract.

2.10.1. Test for tannins

To 2 ml of each stem bark and leaf ethanolic extracts, 2 ml of distilled water was stirred, and few drops of Iron (III) chloride ($FeCl_3$) solution were added. The formation of a greenish or bluish-black precipitate indicated the presence of tannins

2.10.2. Test for saponins

Stem bark and leaf ethanolic extracts (5 ml) each were vigorously shaken with 5 ml of Distilled water in a test tube and warmed. The mixture was then left for observation on the production of the persistent or stable foams indicated the presence of saponins.

2.10.3. Test for flavonoids

To 2 ml of each stem bark and leaf ethanolic extracts in water, dilute sodium hydroxide solution (1 ml) was added. Production of yellow color indicated the positive test or presence of flavonoids.

2.10.4. Test for terpenoids

Stem bark and leaf ethanolic extracts (2 ml) each were dissolved in 2 ml of Chloroform and evaporated to dryness. Concentrated sulphuric acid (2 ml) was then added and heated for about 2 min. A greyish colour indicated the presence of terpenoids.

2.10.5. Test for steroids

To stem bark and leaf ethanolic extracts (2 ml) each, 2 ml of chloroform and 2 ml concentrated sulphuric acid were added; the red colour produced in the lower chloroform layer indicated the presence of steroids.

2.10.6. Test for alkaloids

Stem bark and leaf ethanolic extracts (3 ml) each were stirred with 3 ml of 1% HCl on a steam bath. Turbidity of the resulting precipitate upon addition of Mayer's and Wagner's reagent to the mixture indicated the presence of alkaloids.

2.10.7. Tests for cardiac glycosides

The stem bark and leaf ethanolic extracts (3 ml) each were treated with 2 ml of acetic acid containing one drop of ferric chloride solution. Then, 1 ml of concentrated sulphuric acid was added. A brown ring formed at the interface indicated characteristic features of cardiac glycosides.

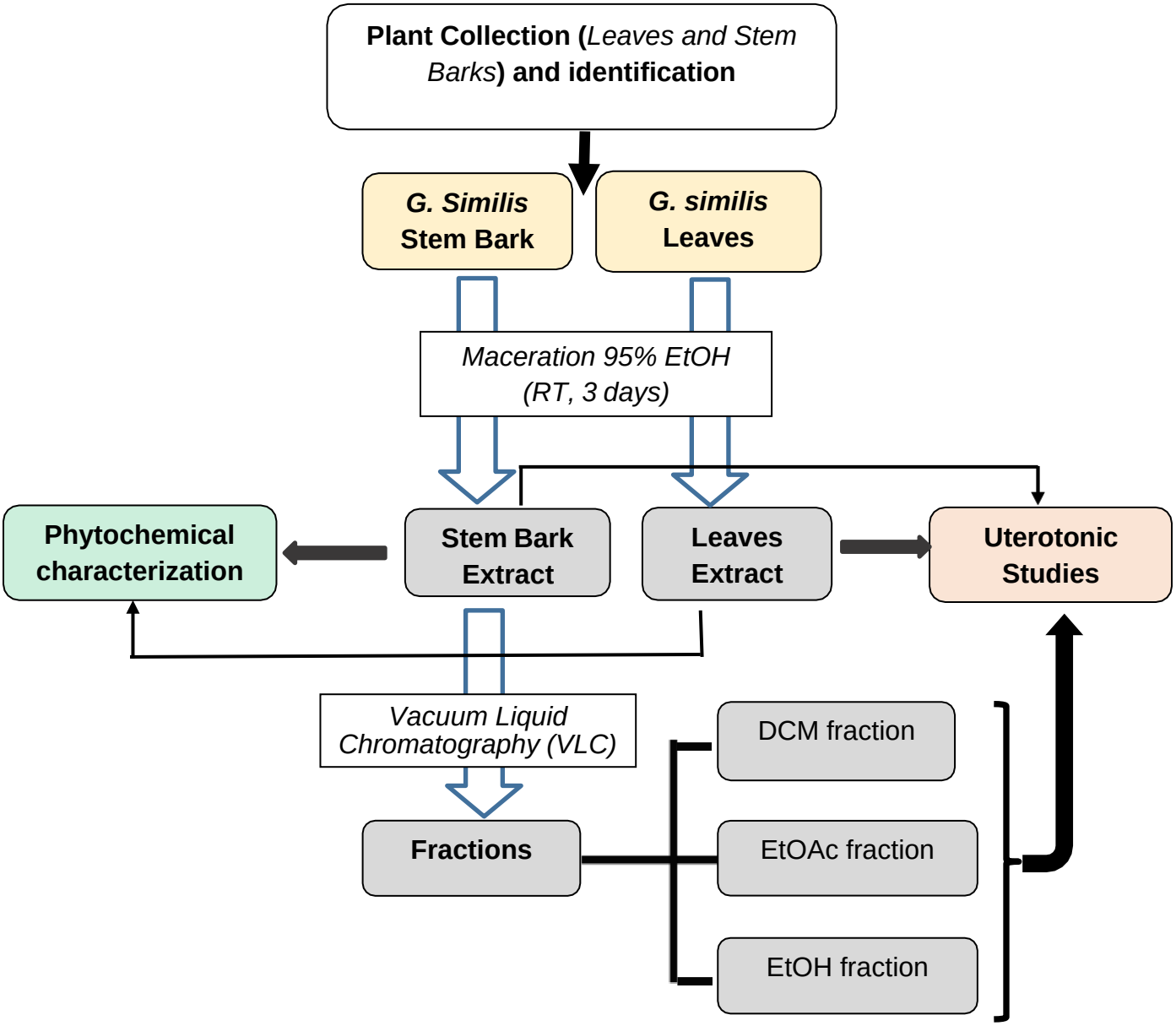


Figure 2: Flow chart of methodology.

2.11. STATISTICAL ANALYSIS

Data on a dose-related increase in force of contraction (as a function of change in length/peak amplitude) of the isolated rat uterus were obtained in triplicate (n=3) for each dose of a particular test and recorded on separate prepared excel data sheets and then transferred to GraphPad Prism software for windows (version 9.0.0, San Diego, CA, USA) for analysis. Results were expressed as mean ± SD, the EC_{50} (concentration needed to produce a 50% maximal response) and E_{max} (maximum achievable response) were computed for each concentration–response experiment. Comparisons among samples was performed by one-way ANOVA followed by post hoc Dunnett's Multiple Comparison test. $p < 0.05$ indicated statistical significance in all cases. The qualitative screening of phytochemicals was reported as positive or negative.

2.12. ETHICAL CLEARANCE

The ethical clearance to conduct this study was granted from Health, Research and Publication Committee of MUHAS with Ref. No. DA.282/298/01.C/ whereas, the use of rats followed the guidelines stipulated in MUHAS-Institutional Animal Care and Use Policy and Procedures (MUHAS-IACUCPP, 2020), Animal Welfare Act, 2008 (United Republict of Tanzania, 2008) and internationally accepted principles for laboratory animal use and care specified in EEC Directive of 1986; 86/609/EEC.

Briefly, the animals were housed in metal cages with enough supply of air, and lighting sequence being 12 h light, 12 h dark in 24 h. Rats were allowed to access food and water *ad libitum*. Used animals were humanely sacrificed and thereafter incinerated.

CHAPTER THREE

3. RESULTS

3.1. EXTRACTS AND FRACTIONS YIELD

The yields of *G. similis* stem bark and leaves crude ethanolic extracts were 86.1 g (2.5%) and 8.0 g (1.6%) respectively. The percentage yield obtained from 26.0 g of the crude stem bark extract of *G. similis* after fractionation by VLC was 95.7%. Since the major fraction was the relatively non-polar one ie. DCM fraction (48.5%), this indicates the presence of high proportion of non-polar compounds in *G. similis* stem bark. The yields of crude extracts and fractions are presented in Table 1 below:

Table 1: Extracts and fractions yields

	Plant material/Crude extract (g)	Crude extract/ Fraction (g)	% Yield
Extraction			
GSLE (*G. similis* leaf extract)	500.00	8.00	1.6
GSBE (*G. similis* stem bark extract)	3500.00	86.10	2.5
Fractionation			
GSBE	26.00		
GSBE-1 (DCM fraction)		12.62	48.5
GSBE-2 (EtOAc fraction)		10.21	39.3
GSBE-3 (EtOH fraction)		2.06	7.9
Recovered GSBE		24.89	95.7
Lost GSBE		1.11	4.3

3.2. UTEROTONIC ACTIVITY AND DEDUCTION OF MECHANISM OF ACTION

3.2.1. UTEROTONIC ACTIVITY

Results of uterotonic activity for oxytocin (OT), crude ethanolic extracts and the respective fractions of the stem bark extract based on isolated rat uterine muscle contraction as a function of change in length/amplitude are presented in **Figures 3-6** and **Tables 2&3**.

In the control (oxytocin), the mean change in length/amplitude due to contraction of the isolated rat uterine smooth muscle tissue recorded was 1.3 (± 0.58) mm, which was observed at 0.04 µg/ml. Meanwhile, the force increased by 8.23 and by 9.77 times, following administration of 0.08 and 0.16 µg/ml oxytocin, respectively, with 0.32 µg/ml oxytocin producing the mean maximum response (Emax) of 13.3 (± 1.16) mm as shown in **Figure 3**.

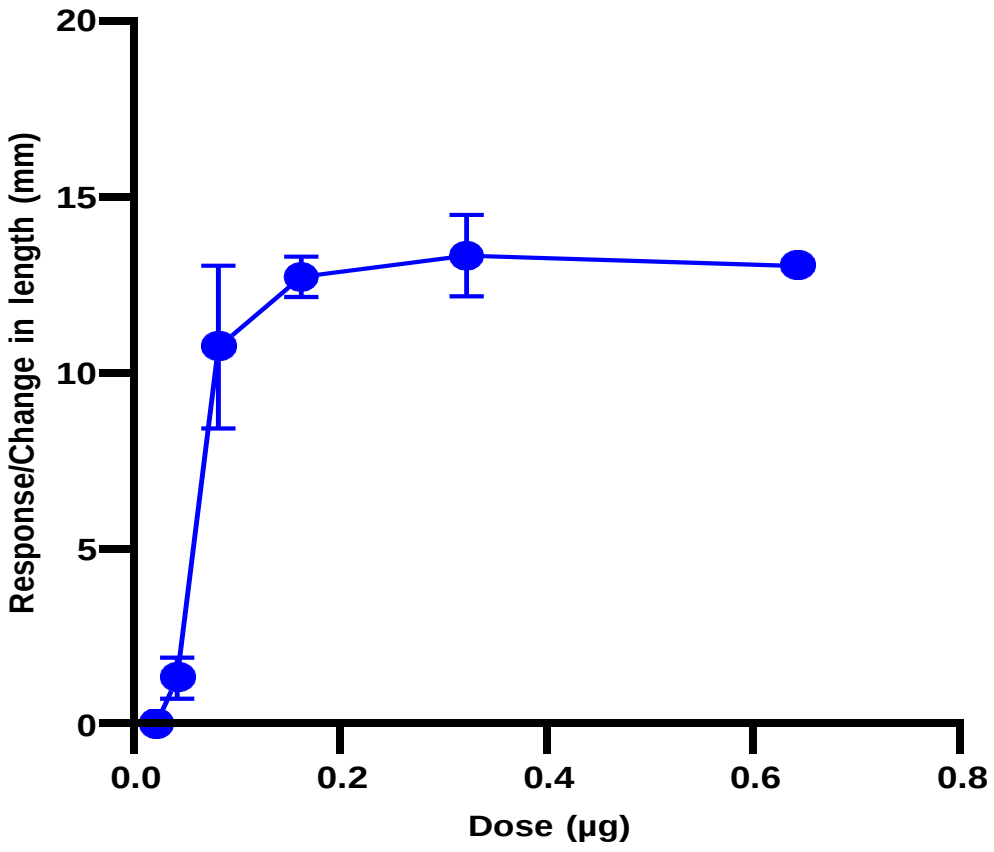


Figure 3: Dose-response curve of oxytocin on the isolated rat uterus

The crude ethanolic extracts used also elicited contractions of varying degree as the concentrations increases, with GSBE at 20 mg/ ml having higher E_{max}, with a mean amplitude 1.30 times greater than the GSLE at 20 mg/ml. GSBE exhibited higher uterotonic activity as compared to GSLE, significant differences ($p < 0.05$) were observed in the EC_{50} and E_{max} in **figure 4** and **tables 2 and 3**.

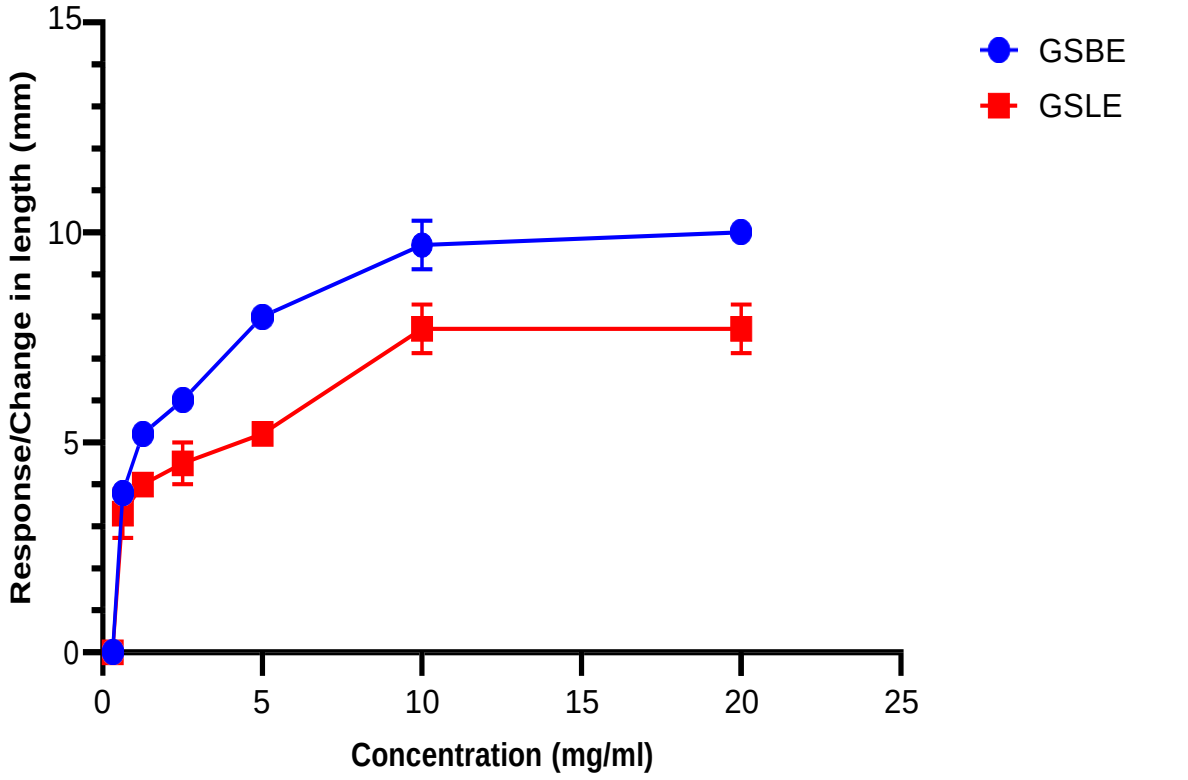


Figure 4: Concentration-response curves of crude ethanolic extracts on the isolated rat uterus

Also, the fractions from GSBE elicited contractions of varying degree as concentrations increases, with 20 mg/ml GSBE-3 having the greatest E_{max}, with a mean amplitude 1.33, 2.00 and 2.41 times greater than the GSBE-2, GSBE and GSBE-1 at 20 mg/ml, respectively. GSBE-3 had shown the highest efficacy in uterotonic activity, statistically significant difference in the EC_{50} and E_{max}, when compared to the GSBE, GSBE-1 and GSBE-2 ($p < 0.05$) as shown in **figure 5** and **tables 2 and 3**.

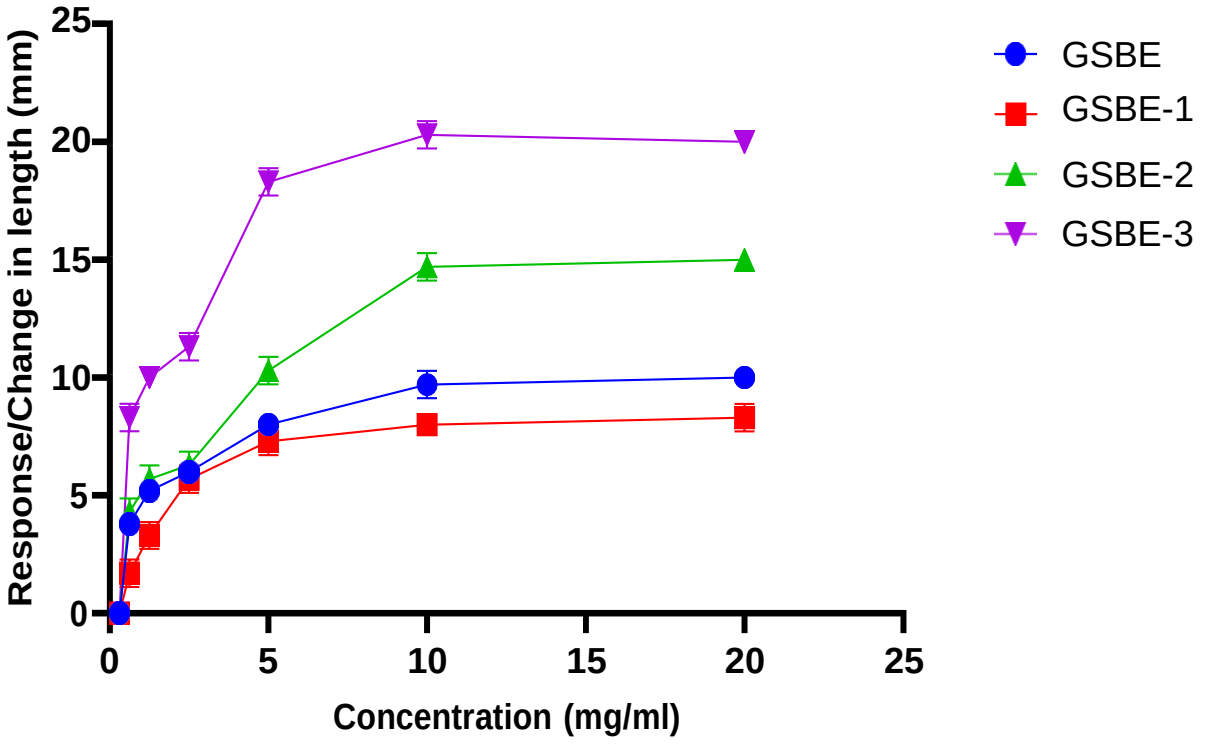


Figure 5: Concentration-response curves of stem bark ethanolic extract and fractions on the isolated rat uterus

When the relative potency of crude extracts and fractions were compared in reference to oxytocin, the gold standard uterotonin, GSBE-3 at 20 mg/ml was found to be 2 times more potent than 20 mg/ml GSBE in the stilbestrol treated non-pregnant rat uterus. The dose of GSBE-3 that produced the Emax (20 mg/ml) showed a mean amplitude greater than that of 0.08 µg/ml oxytocin. The smallest EC_{50} value (0.06 µg/ml) oxytocin implies that, it is more potent than both crude extracts and fractions. This is shown in **Tables 2, 3 and Figure 6**.

Table 2: EC_{50} values of the test drugs and positive control drug

Drugs	**EC_{50}**
GSLE	1.42 mg/ml
GSBE	1.36 mg/ml
GSBE-1	1.54 mg/ml
GSBE-2	1.45 mg/ml
GSBE-3	1.31 mg/ml
Oxytocin	0.06 µg/ml

Table 3: Comparisons of rat uterine contractile effect of test drugs with a positive control

Uterine contraction of test drugs with positive control *(Dunnet test)*	*p*-value
*Oxytocin vs. GSLE	0.0127
Oxytocin vs. GSBE	> 0.05
Oxytocin vs. GSBE-1	> 0.05
Oxytocin vs. GSBE-2*	0.0008
Oxytocin vs. GSBE-3*	< 0.0001

**Represents statistical significance difference of test drugs with positive control drug*

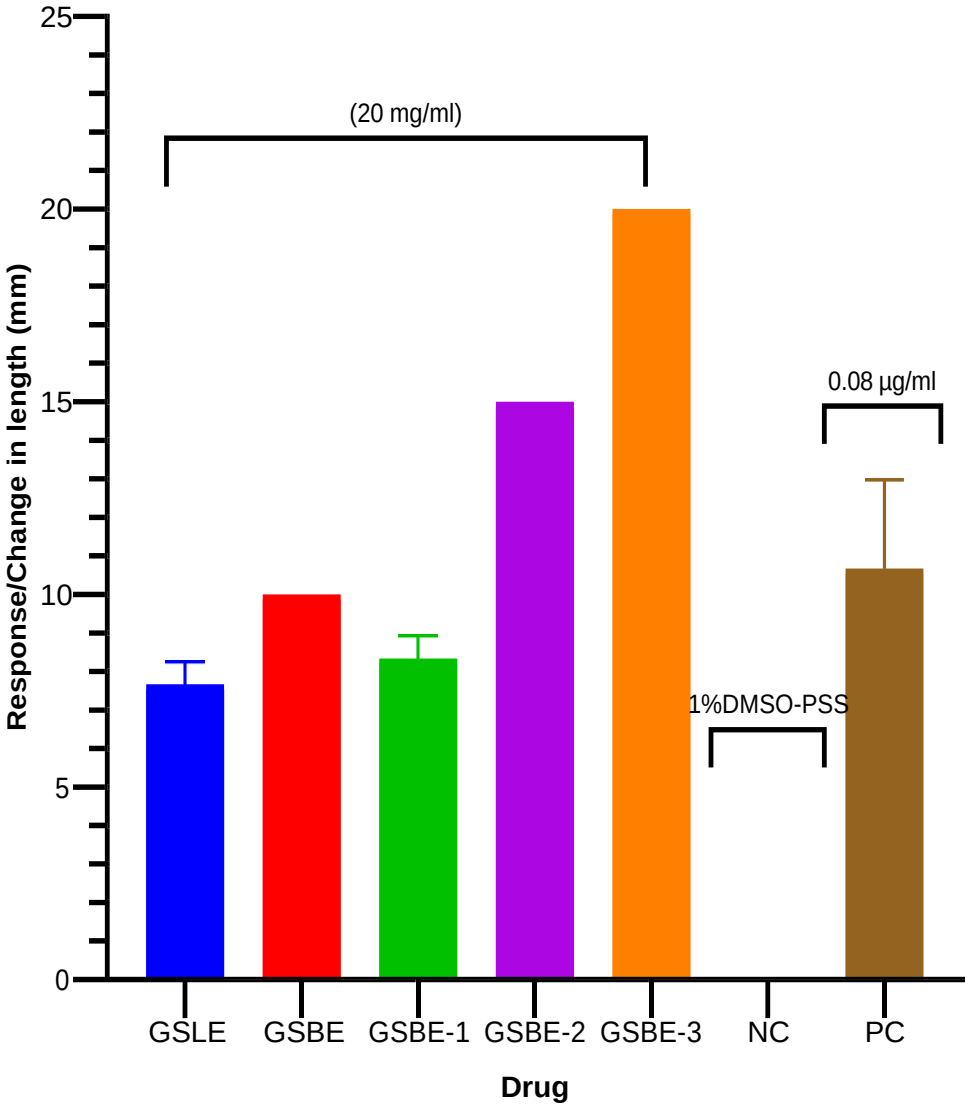


Figure 6: Uterotonic responses of a stated dose of crude ethanolic extracts, fractions, negative control (NC) and positive control (PC) on the isolated rat uterus

3.2.2. PHARMACOLOGICAL ACTION

In deduction of the possible mechanism of action through uterine contraction inhibition, significant differences ($p < 0.05$) were observed in the EC_{50} and E_{max} of GSBE-3 in the presence of calcium channel blocker-nifedipine (NF), prostaglandins receptor antagonist-meloxicam (MX) and histamine receptor antagonist-diphenhydramine (DP). There was no significant difference in the EC_{50} and E_{max} of GSBE-3 alone and in the presence of muscarinic receptor antagonist-atropine (AT). (**See table 4 and figures 7-9**).

Pre-treating the tissue with either nifedipine, meloxicam or diphenhydramine before administering the extract showed an inhibitory effect while in the presence of atropine no effect was observed, an extract showing a probable interference with calcium channels, stimulation of prostaglandins synthesis and/or activation of histamine receptors in utero.

Table 4: EC_{50} values of GSBE-3 alone and with some uterine receptor antagonists

Extract/drugs	**EC_{50} (mg/ml)**
GSBE-3 alone	1.31
GSBE-3 + NF (2.50 nM)	2.43*
GSBE-3 + NF (20.50 nM)	-
GSBE-3 + MX (1.45 nM)	1.69*
GSBE-3 + MX (14.25 nM)	-
GSBE-3 + DP (4.45 nM)	1.94*
GSBE-3 + DP (44.50 nM)	-
GSBE-3 + AT (20.00 nM)	1.30
GSBE-3 + AT (100.00 nM)	1.32

*"–"=EC_{50} could not be computed with available data due to extreme inhibition. *Represents statistical significance difference in EC_{50} values of GSBE-3 alone and in the presence of antagonists* ($p < 0.05$)

Effect of nifedipine on GSBE-3-induced uterine contraction.

Nifedipine significantly depressed ($p < 0.05$) the E_{max} of the GSBE-3 by 0.85 times **(Figure 7)** and also significantly increased ($p < 0.05$) the EC_{50} by 1.12 mg/ml respectively **(Table 4)**.

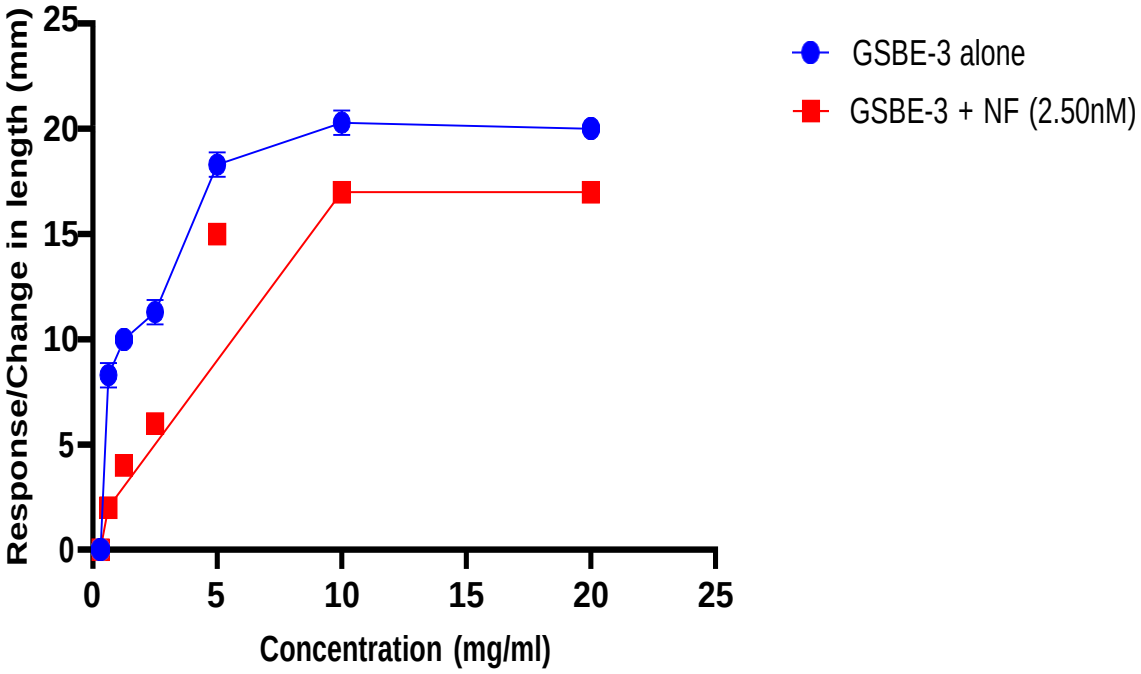


Figure 7: Concentration–response curves of GSBE-3 in the presence of nifedipine (NF).*p < 0.05 compared to GSBE-3 alone

Effect of meloxicam on GSBE-3-induced uterine contraction

Meloxicam significantly depressed ($p < 0.05$) the E_{max} of GSBE-3 by 0.80 times **(Figure 8)** and also significantly increased ($p < 0.05$) the EC_{50} by 0.38 mg/ml respectively **(Table 4)**.

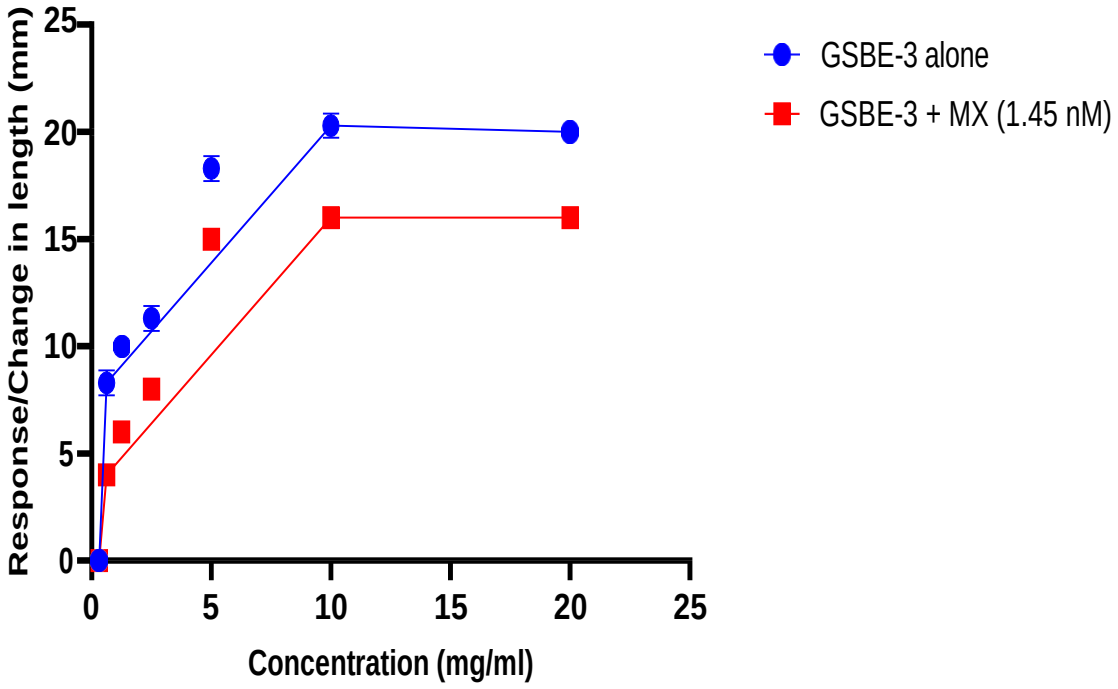


Figure 8: Concentration–response curves of GSBE-3 in the presence of meloxicam (MX).*p < 0.05 compared to GSBE-3 alone

Effect of diphenhydramine on GSBE-3-induced uterine contraction

Diphenhydramine significantly increased ($p < 0.01$) the EC_{50} by 0.63 mg/ml **(Figure 9)** and had significant effect on the E_{max} of the GSBE-3 reduced by 0.75 times **(Table 4)**.

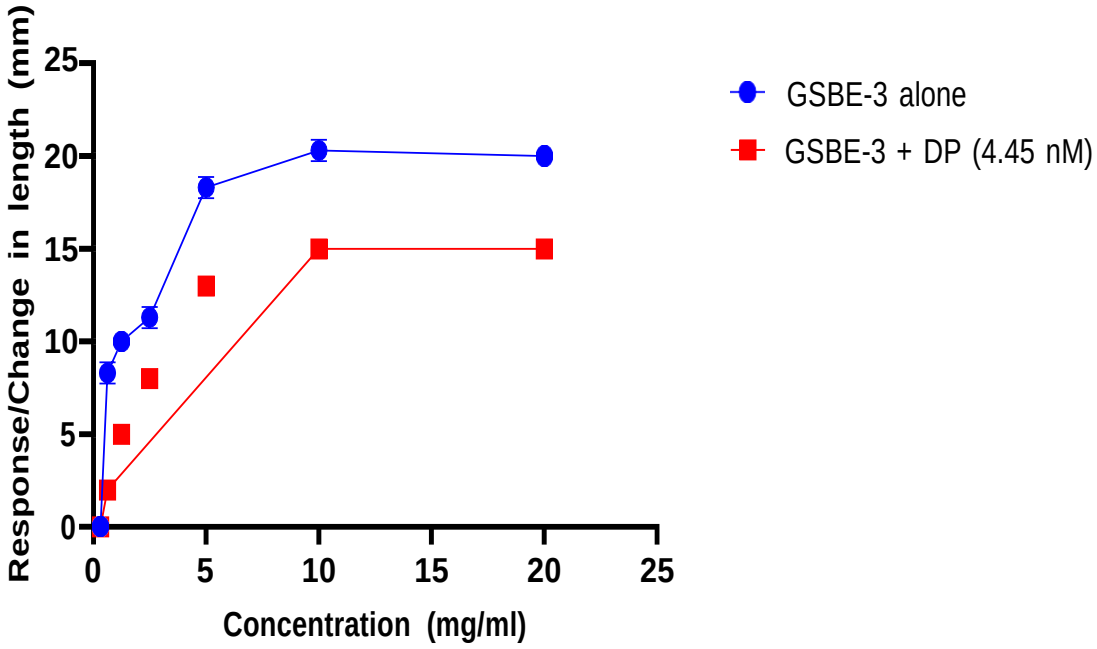


Figure 9: Concentration–response curves of GSBE-3 in the presence of diphenhydramine (DP).*$p < 0.05$ compared to GSBE-3 alone

Effect of atropine on GSBE-3-induced uterine contraction

No significant inhibition of the GSBE-3 was observed in the presence of AT, this was evident by the lack of significant increase in the EC_{50} **(Table 4)**.

3.3. PHYTOCHEMICAL SCREENING

Standard phytochemical tests for the crude ethanolic extract of *G. similis* stem bark and leaves showed the presence of tannins, flavonoids, steroids and saponins. Other phytochemical classes could not be detected. Results are summarized in **Table 5.**

Table 5: Phytochemical Groups of *G. similis* Crude Ethanolic stem bark and leaf Extracts

Phytochemical group	**Chemical test**	**Results**	
		Stem barks	**Leaves**
Alkaloids	Mayer's and Wagner's test	Negative	Negative
Cardiac glycosides	Keller - Kiliani test	Negative	Negative
Flavonoids	Dilute NaOH test	Positive	Positive
Saponins	Foam test	Positive	Positive
Steroids	Salkowski Test	Positive	Positive
Tannins	Ferric Chloride test	Positive	Positive
Terpenoids	Salkowski Test (modified)	Negative	Negative

Where; **'positive'** means **"detected/present"** and **'negative'** means **"not detected/absent"**

CHAPTER FOUR

4. DISCUSSION

The yields of crude ethanolic extracts from the stem bark and leaf were 2.5% and 1.6% respectively. *Grewia similis* stem bark crude extract contained non-polar compounds in higher amount i.e GSBE-1 (48.5%) as compared to relatively polar fractions giving a similar pattern observed from a study done in Kenya on the same species using roots (Muithya, 2010).

This study has shown a scientific proof on the uterotonic effect of crude ethanolic extracts of *G. similis* leaves and stem bark which supports ethnomedical uses of this plant in the highlands of North Western Tanzania - Lushoto district, Tanga region. The results are in line with those obtained from related species, including; barks and woods of *G. occidentalis* L., and roots and barks of *G. bicolor* Juss reported in previous studies (Jaspers *et al*., 1986; Mulholland *et al.*, 2002). Together with other *Grewia* species, *G. similis* can serve as the potential source of providing uterotonic agents for drug development.

Oxytocin is a known potent uterotonic drug that is used clinically (Devikarani and Harsoor, 2010). In this study it was employed as a positive control for uterotonic activity because it is the drug of choice for induction or acceleration of labour and in the management of postpartum hemorrhage. The efficacy of stem bark extract was significantly higher compared to that of the leaf extract ($p < 0.05$) as shown in **Figure 4**. However, oxytocin potency is far much higher compared to both crude extracts and GSBE fractions. In part, this could be explained by the fact that, oxytocin is a pure compound whereas crude extracts as well as tested fractions are composed of a mixture of active and non active compounds (Ie and Zam, 2008). Oxytocin produced a maximum dose-response (E_{max}) at the concentration of 0.32 µg/ml (0.28-0.34 µg/ml) with EC_{50} of 0.06 µg/ml (0.033-0.098 µg/ml) while stem bark and leaf ethanolic extracts produced maximum responses at the concentration of 20 mg/ml (20.012-20.041mg/ml) with EC_{50} of 1.36 (1.080-1.702) and 1.42 mg/ml (1.048-1.923 mg/ml) respectively **(Figures 3 & 4)**. In the comparison of induction of uterine contraction of dose-response of crude ethanolic extracts with that of oxytocin at stated doses/concentrations, the response of oxytocin is higher than that of GSLE ($p < 0.0127$) but there was no significant difference with that of GSBE ($p > 0.05$). GSBE (20 mg/ml) produced response which is equivalent to that produced by 0.08 µg oxytocin **(Figure 6, Table 3)**, a gold standard uterotonin (Sheldon *et al*., 2012) in non-pregnant rats.

Fractions obtained from the crude ethanolic extracts of the stem bark also exhibited uterotonic activity of various levels (GSBE-3 > GSBE-2 > GSBE-1) as indicated in **Figure 5** and **Table 2**. GSBE-3 showed higher efficacy in uterotonic activity when compared to the GSBE, GSBE-1 and GSBE-2 ($p < 0.05$) as shown in **Figure 5** and **Tables 2** and **3**. In the comparison of induction of uterine contraction of dose-response of fractions with that of oxytocin at stated doses/concentrations, the response of oxytocin is much similar to that of GSBE-1 ($p > 0.05$) but less than that of GSBE-2 ($p < 0.0008$) and GSBE-3 ($p > 0.0001$) **(Table 3)**. The response of GSBE-3 (20 mg/ml) is 2 times more than oxytocin at 0.08 µg/ml as shown in **Figure 6**. Since high activity was obtained from GSBE-3, this indicates that, the polar compounds present in *G. similis* stem bark were most involved in the activity. In agreement with these findings is the work on *G. occidentalis* using the most polar fraction obtained by supercritical fluid extraction (SFE) gave similar results (Mulholland *et al.*, 2002).

Regarding the possible mechanism of action(s) by which *G. similis* stem bark ethanolic extract contracts the rat uterus, the findings of this study suggest that their uterine contractile effect was mediated via calcium channels, prostaglandins and histamine receptors. These mechanisms were confirmed by changes in EC_{50} and inhibition of E_{max} when the uterus was treated with antagonist prior to test drug administration **(See Figures 7-9, Table 4)**.

Due to the antagonism of GSBE-3 by nifedipine (an L-type calcium receptor antagonist), the possible involvement of receptor- and voltage-operated calcium channels (ROCs &VOCs) in rat uterine contraction is suggested. The contractile effect of the GSBE-3 was reduced and completely abolished after nifedipine 2.50 nM and 20.50 nM application respectively. The findings suggest that GSBE-3-induced uterine contraction was mediated via the L-type calcium receptor. However, it is also possible that GSBE-3 may have a direct receptor effect which is linked via second messengers to calcium and hence calcium channels (Michel *et al*., 2001).

Addition of meloxicam results in inhibition of "synthesis" of the uterine active prostaglandins (Landen *et al*., 2001). The marked attenuation of rat uterine contraction by meloxicam, suggests that GSBE-3 contracts the uterus directly or indirectly by stimulating the synthesis and release of uterotonic prostaglandins or there might be a continuous synthesis of prostaglandins in the uterus

which sensitizes the uterus to GSBE-3 (Vane and Williams, 1973). There is also a possibility that meloxicam can antagonize the effect of GSBE-3 produced following it binding to the oxytocin receptors, this is due to a high number of this receptor expression in the uterus. Previous studies have shown that of all the receptors reported to be present in the uterus, the oxytocin receptor expression in the uterus is the highest (Sanborn, 2001; Grigsby *et al*., 2006).

Diphenhydramine in this study also inhibited GSBE-3-induced rat uterine contractions. Since diphenhydramine, like other H_1-receptor antagonists, is a reversible competitive inhibitor of the interaction of histamine or other histamine receptor agonists with H_1-receptors. This action leads to inhibition of most responses of smooth muscle to histamine and histamine receptor agonists (Brown and Roberts, 2001). The presence of histamine H_1- and H_2- receptors in the uterus are well documented where H_1-receptor mediates uterine smooth muscle contraction (Fleisch *et al*., 1987). Though diphenhydramine has muscarinic blocking effect (Hoffman, 2001), the possibility that its inhibition of GSBE-3-induced rat uterine contraction might be through blockade of muscarinic receptors has been delineated since atropine failed to inhibit the fraction's response.

Atropine, a naturally occurring alkaloid and a well known non-competitive muscarinic receptor antagonists (Palczewski *et al*., 2000) was shown in this study, to have no effect on the responses of the uterus to GSBE-3. This suggests that the extract does not contract the uterus via activation of muscarinic receptors in the uterus.

From the observed results on the inhibition of the GSBE-3's activity by several receptor antagonists suggests that there are several possible compound classes present in the plant that are responsible for the observed activity on the uterus. The plant extracts induce/increase the contraction of rat uterine smooth muscle via multiple membrane receptors, this is supported by the phytochemical analysis as described below.

Phytochemical analysis of the plant *G. similis* stem bark and leaves revealed the presence of tannins, flavonoids, saponins and steroids. The findings are in agreement with previous studies in which same phytochemical groups were detected from related plants of the same genus/family which also demonstrated uterotonic/oxytocic properties. These include; *Grewia bicolar* Juss [roots and barks] (Jaspers *et al*., 1986), *Grewia occidentalis* L. [barks and woods] (Mulholland *et al*., 2002), *Grewia damine* [leaves] (Jayasinghe *et al*., 2004).

The most polar fraction GSBE-3 demonstrated higher uterotonic activity compared to less polar fractions of the crude extract. Since tannins and flavonoids are polar in nature, they are likely the ones which have contributed to the uterotonic activity of this fraction. This speculation is based on a previous study on tannins from various plants that showed uterotonic effect by affecting calcium availability for uterine tissue and cardiac muscle contraction (Calixto *et al*., 1986; Polya *et al*., 1995) and the fact that, flavonoids are known to act directly on oestrogen receptors leading to uterine contraction (Revuelta *et al*., 1997).

Various flavonoids such as vitexin, isovitexin and conififeraldehyde have been isolated from the leaves and stem barks of some *Grewia* species. Conififeraldehyde demonstrated moderate uterotonic activity when compared to linolenic acid, both of which were isolated from *Grewia occidentalis* (Mulholland *et al*., 2002; Jayasinghe *et al*., 2004).

Previous work reported the presence of 3β-stigmasterol and 3β-sitosterol from roots of *G. similis* (Muithya, 2010), leaving the possibility of having the same compounds in leaves and stem of *G. similis.* Also, 3β-sitosterol as a known uterotonic agent (Promprom *et al*., 2010) could in part contribute to the observed uterotonic activity together with other phytochemicals therein.

Compound(s) belonging to the detected phytochemical groups are likely the ones that have produced the observed uterine contraction in this study but this does not exclude other phytochemical groups that could not be detected due limitations such as insensitive chemical tests/presence in very low amounts. It is also important to note that, crude extracts containing a mixture of several compounds, a particular activity could result from a single compound/related compounds of a certain phytochemical group, or compound(s) from various phytochemical groups.

CHAPTER FIVE

5. CONCLUSION AND RECOMMENDATION

5.1. CONCLUSION

Evaluation of uterotonic activity in albino rats for 95 % ethanolic crude extracts and fractions of *G. similis* stem barks showed uterotonic activity. Fractionation was done and it was found that both polar and non polar fractions of the stem bark extracts exhibited uterotonic properties (most polar fraction i.e, GSBE-3, being the most active). The pharmacological effects/possible mechanisms results suggest that the stimulation of uterine contractility by the stem bark ethanolic fraction of *G. similis* may arise from the interference with calcium channels, stimulation of prostaglandin synthesis and/or activation of histamine H_1- receptors in utero.

Phytochemical screening of the crude ethanolic extract of *G. similis* revealed the presence of tannins, flavonoids, saponins and steroids. These types of phytochemicals have been reported to have uterotonic activity. The use of this plant to induce or augment labour by traditional health practitioners may be due to the contractile effect of its active phytochemical constituents.

5.2. LIMITATION AND MITIGATION

The limitation of the study is from estrous induction of the laboratory animals using DES, as it is carcinogenic, poor absorption from the site of injection especially when injected wrongly and the availability of the drug in our setting is low. Coconut oil and < 30% ethanol were used for drug reconstitution and increasing absorption of the drug from the site of injection.

5.3. RECOMMENDATION

From this study, uterotonic activity, possible mechanism of action, identification of the most active fraction and phytochemical groups have been achieved; hence further studies are recommended for the isolation of phyto-constituents responsible for the reported activity, safety evaluation and formulation of standardized herbal products of *G. similis*. The isolated phytoconstituents can be considered as potential lead compounds for development of uterotonic medicine (s)

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APPENDIX

INVESTIGATION TOOL

Table 3: Observation Chart for Uterotonic activity Studies

DATE: ……

ANIMAL TISSUE: Rat uterus

TEST DRUG/STANDARD DRUG: ……

SOLVENT…….

REPEATABILITY TEST (n): ……

S/N	Volume of the stock solution (ml)	Concentration of the stock solution (mg/ml)	Final concentration in organ bath (mg/ml)	Mean uterine contraction (as a function of change in length/peak amplitude) in mm	S.D
1					
2					
3					
4					
5					