INVESTIGATION OF UTEROTONIC ACTIVITIES, ACUTE TOXICITY IN RATS AND PHYTOCHEMICAL COMPOSITION OF *HYDNORA ABYSSINICA* AND *UVARIODENDRON ANISATUM* EXTRACTS

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DEDICATION

I dedicate my thesis to my family and many friends. I wish to express gratitude to my loving wife and children whose words of encouragement and push for tenacity is fresh in my mind.

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LIST OF ABBREVIATIONS

ALP	Alkaline phosphatase
ALT	Aminotransferease
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
BAUEC	Biosafety, Animal use and Ethics Committee
FVM-BAUEC	Faculty of Veterinary Medicine Biosafety, Animal Use, and Ethics Committee
i.m	Intramuscular
Iu	International units
LAMART	Department of Land Resource, Management, and Technology
LD_{50}	The amount of drug, when given all at once, causes death in 50% of a group of test animals
MMR	Maternal death rate
NACOSTI	National Commission for Science, Technology, and Innovation
OECD	Organisation for Economic Co-operation and Development
PPH	PostPartum Hemorrhage
SEM	Standard Error of the Mean

ABSTRACT

Uterotonic drugs are used to manage postpartum bleeding in the mainstream medical systems. However, global maternal mortalities due to postpartum complications are still high due to lack of timely interventions, qualified staff, and total costs of hospital-based deliveries. Traditional medicine systems provide primary health care alternatives for the management of postpartum hemorrhage. Over 80% of cultural societies in low-income Countries use plant preparations in traditional medicine with unknown potency and safety profiles. In light of this background, the current study investigated uterotonic activities, toxicity in rats, and phytochemical composition of *Hydnora abyssinica* and *Uvariodendron anisatum* extracts. The plants were collected from Embu County, where they are used in removing retained placenta and management of postpartum hemorrhage. Laboratory experimental design was adopted and the extracts were macerated and extracted using ethanol and water. Uterus for uterotonic activity assay was isolated from primed mature female Wistar rats. A piece of the uterus (2 cm) was mounted on an organ bath and exposed to De Jalon's solution as a negative control and oxytocin (positive control), and extracts with concentrations varying from 0.5 to 4.0 mg/ml were added. Acute oral toxicity studies were done following the OECD 423 guideline and phytochemical screening by adopting the standard phytochemical procedure. The study was approved by the Faculty of Veterinary Medicine Biosafety, Animal Use, and Ethics Committee of the University of Nairobi, and the National Commission for Science, Technology, Innovation. Data obtained from the uterotonic activities was analysed using GraphPad Prism Version 8.0.1 software and was expressed as a percentage increase or decrease in the mean ± standard error of the mean (SEM) relative to the controls. The findings of acute oral toxicity of *H. abyssinica* rhizome and *U. anisatum* root extracts were expressed using a dose that causes a mortality of 50% of study animals (LD_{50}). Additionally, the phytochemical components of the plants in this study were tabulated. It was found that the

extracts of *H. abyssinica* rhizome and *U.anisatum* root had uterotonic activity. The ethanol extracts of *H. abyssinica* rhizome demonstrated significantly high activity compared with that of the water extract at $p < 0.001$***. On the other hand, the uterotonic effect of *U. anisatum* root water extracts was higher than that of ethanol. A single dose of the *H abyssinica* rhizome and *U. anisatum* root at 2000 mg/kg did not cause mortality of the tested Wistar rats. Alkaloids, glycosides, phytosterols, triterpenes, diterpenes, proteins, phenols, flavonoids and fixed were present in *H. abyssinica* and *U. anisatum.* However, saponins and volatile oils were only present in *U. anisatum.* The findings from this study provide scientific evidence which is useful in validating the use of *H. abyssinica* and *U. anisatum* extracts in the removal of retained placenta.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Research

Plant use in complementary medicines has remained significant in primary health care throughout human history (Dhami, 2013). Various historical and religious literature reveal the use of medicinal plants. The ancient records include the Hindu religious writings (The Veda), which was composed between the 2nd and 1st Millennium BC (Gewali, 2008). Subsequent writings include the *Chinese Materia Medica* (1100 BC) (Saad *et al*., 2017), the Bible (1400 BC) (Krymow, 2012), Egyptian *Ebers papyrus* (1550) (Dhami, 2013) to the present Pharmacopoeia descriptions which include British Herbal Pharmacopoeia, as well as African herbal Pharmacopoeia (Brendler et al., 2010). While there is continuous improvement in health care services and products with mainstream medicine, over 80% of individuals in developing states continuously depend on therapeutic herbs for initial healthcare necessities, including those arising during pregnancy (Ahmad & Ahmad, 2018; Zamawe et al., 2018).

1.2 Problem Statement

Maternal mortality rates are persistently elevated in Low and Middle Income Countries (LMIc), with a maternal death rate (MMR) estimated at 239 for every 100,000 live deliveries. This is due to inadequate professional services, poor timing, and under exploitation of available medical interventions (Riang'a *et al*., 2018; World Health Organisation, 2015) as well as the high cost of medicinal care. There are many delivery complications which lead to maternal deaths, illnesses and postpartum hemorrhage (Fukami et al., 2019). Some of the underlying factors include uterine atony, retained placenta, adherent placenta, vaginal hematomas, cervical tears as well as uterine angle lengthening (Edhi *et al*., 2013). Despite the

Government of Kenya efforts to achieve Universal Health Care and Sustainable development goal three, maternal and neonatal mortalities remain high in Africa (World Health Organisation, 2015). In Kenya about 40% of the deliveries occur without professional assistance (Gitobu, 2018). In addition, neonatal maternal mortality ratio and neonatal mortality rates are estimated at 362/100,000 live deliveries, as well as 22/1000 live births, correspondingly (Gitobu et al., 2018). Potent and safe plant extracts are used as alternative medicines in order to promote maternal and neonatal health in Kenya, especially in rural areas (Gachathi, 2007; Kaingu et al., 2011; Kokwaro, 2009).

1.3 Justification of the study

Plant herbal products continue to be used as complementary alternative medicines in the care of pregnant women globally (Gruber & Brien, 2012). However, the effectiveness and safety of many plants employed in traditional medicine systems is yet to be validated (Kupittayanant et al., 2014). The phytochemical compounds responsible for various gynecological benefits therefore require to be isolated with the intent of drug development (Kupittayanant et al., 2014). The current study seeks to evaluate uterotonic activity, toxicity in rats and the phytochemical composition of *Hydnora abyssinica* and *Uvariodendron anisatum.* The two plants have been known to have gynecological benefits in some Kenyan communities' traditional medicinal systems (Gachathi, 2007; Kaingu *et al*., 2011; Kaingu *et al*., 2017). Findings from the study will be of benefit and great interest to researchers, health care practitioners and other stakeholders in the Health Sector.

1.4 Study Objectives

1.4.1 Overall Objective

The overall research objective of the study was to investigate the uterotonic activities and toxic effects of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts in Wistar rats and determine phytochemical constituents of *Hydnora abyssinica* and *Uvariodendron anisatum* plants.

1.4.2 Specific Objectives

i. To investigate uterotonic activities of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts using the uterus of Wistar rats.
ii. To evaluate acute oral toxic effects of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts in female Wistar rats.
iii. To determine the phytochemical composition of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts.

1.5 Null Hypothesis

i. *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts do not have uterotonic effect.
ii. The extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root do not exhibit acute oral toxicity effects on Wistar rats.
iii. The extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root do not contain phytochemical groups of substances

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional medicine and maternal health

Diverse groups of healing systems which are not considered to be part of mainstream medicine are referred to as traditional medicine and complementary alternative medicine in developing and developed countries respectively (Shewamene et al., 2017). The term traditional medicine is used interchangeably in many countries. Traditional medicine is popular in primary health care used by about 80% of the population in developing countries (World Health Organization, 2019). In many low and middle-income countries, traditional medicines are used to manage the health of majority of women during pregnancy, childbirth and postpartum period. This is the case where the official health care systems are underperforming due to constrained resources (Esamai et al., 2017; Mudonhi & Nunu, 2021).Traditional medicine account for over 60 % of Maternal primary health care and ensuring the safety and efficacy of traditional medicines and services therefore supporting health individuals and families (Chotchoungchatchai et al., 2020; Sarmiento et al., 2016).

2.2 Postpartum hemorrhage

Postpartum hemorrhage (PPH) describes post-delivery bleeding. It is classified into primary and secondary PPH. Primary PPH is characterized by loss of approximately 500 ml, whereas the secondary PPH results in loss of more than 1000 Mls of blood within 24 hours of normal vaginal and cesarean delivery. (Likis et al., 2015). Globally, it is the principal reason for maternal mortalities, as well as morbidities in developing countries. However, most of the PPH complications can be avoided using timely detection and aggressive treatment in mainstream Health care systems. Uterotonics, usually 10 IU of oxytocin (i.m), are employed

clinically in PPH management. *Misoprostol* is an alternative drug administered orally, which can also be used to manage PPH cases. Notably, *Misoprostol* has known side effects (Morris et al., 2011).

2.3 Medicinal plants that manage post-partum hemorrhage

Various cultural societies have plant-based Medicines used to manage delivery-associated complications in alternative Medicine systems (Hajj & Holst, 2020; Mudonhi et al., 2021; Tsitsi & Precious, 2016) . Some of the preparations known to have uterotonic activities are reported to decrease post-partum hemorrhage (PPH) and other birth related illnesses in Africa. In sub-Saharan Africa the plants commonly used to manage post-partum hemorrhage related complications include *Agapanthus africanus*, *Clivia miniata*, *Gunnera perpensa*, *Monechma ciliatum*, *Rhoicissus tridentata*, and *Spondias mombin, Azadirachta indica, Senna occidentalis Sida acuta* and *Cola gigantea* (Hajj & Holst, 2020; Tripathi, Stanton, & Anderson, 2013).

In Kenya, over 100 plants have been documented in different communities to manage delivery complications. Some of the plants used to remove retained placenta and manage of post-partum hemorrhage are *Basella alba*, *Cleome gynandra* (Jeruto et al., 2008), *Ricinus communis*, *Euclea divinorum*, *Ocimum americanum*, *Strychnos henningsii*, *Steganotaenia araliaceae*, *Clausena anisatum*, *Barleria eranthemoides*, *Lippia javanica*, *Fagaropsis hildebrandtii*, *Croton macrostachyus*, *Pappea capensis*, *Aspilia mossambicensis*, *Hoslundia opposite*, *Fuerstia africana*, *Bidens Pilosa*, *Vernonia glabra*, *Withania somnifera*, *Boscia angustifolia* and *Aloe secundiflora*, (Gachathi, 2007; Kaingu et al., 2011; Kokwaro, 2009).

2.4 *Hydnora abyssinica* A. Braun (Hydnoraceae)

2.4.1 General description of *Hydnora abyssinica*A. Braun

As indicated by Tennakoon et al. (2007), *Hydnora abyssinica* A. Braun Schweinf (Fig. 1) is identical to *H. johannis* Becc. Nouv., and *H. solmsiana* Dinter. It is called *Muthigira* in Kikuyu, *Mũtũmũra Nthí* in Embu/Mbeere (Onyancha et al., 2019) and *Kimela* in Kamba (Kaingu et al., 2011a) . The herb is parasitic and has a characteristic foul odor. Prominent features include 10-15 cm in height, with a typical attachment on *Acacia nilotica* roots, as well as on multiple other *Acacia* species. The visible parts above the ground are the floral segments. The rhizome's appearance depicts a thick, rigid, and black root with a dark brown color. Additionally, the tuber has a warty appearance that attaches to the host's root via haustoria (Gachathi, 2007).

2.4.2 Biological activity of *Hydnora abyssinica*A. Braun

Reports suggest *Hydnora abyssinica* has a mild antioxidant property (Onyancha *et al*., 2015). Additionally, cytotoxicity research indicates the plant's extracts inhibit mouse fibroblast proliferation, (3T3) epidermoid carcinoma typically affecting the oral region in humans (KB), as well as normal human fetal lung (MRC5) cell lines (Waleed *et al*., (2009); Yagi *et al*., (2012). Concurrently, *in vitro*, *H. abyssinica* extracts have been found to have high antimicrobial and antifungal activities (Saadabi and Ayoub, 2009; Yagi *et al*., 2012; Ndwigah *et al*., 2014).

2.4.3 Phytochemistry of *Hydnora abyssinica*A. Braun

Hydnora abyssinica rhizome extracts contain tetra decanoic acid catechin, tyrosol, benzoic acid, cirsiliol, sigmasterol, oleic compounds, and myristic acid. Other compounds present include palmitic acid, trans-3′5-dihydroxy-4′7-dimethoxy dihydro flavonol, vanillin, as well as protocatechuic acid (Waleed *et al*., 2009; Yagi *et al*., 2012; Waleed *et al*., 2015).

Moreover, various phytochemical elements are also present in the plant, encompassing phenols, tannins, and proanthocyanins. At the same time, flavonoids, mucilage, and other compounds, such as alkaloids, glycosides, triterpenes, as well as sterols are present (Waleed *et al*., 2009; Onyancha *et al*., 2015).

2.4.4 Ethnobotanical uses of *Hydnora abyssinica* A. Braun

The fresh part of *Hydnora abyssinica* flower bud (calyx) is eaten as a vegetable. (Maundu, 1999). Various infusions and decoctions extracted from the rhizome are applied to treat various illnesses, such as evil eyes and Cholera. Moreover, the extracts are used in treating sore throat, oral thrush and are significantly effective against amoebic dysentery. Other applications include treating diarrhoea, stomach upsets, pneumonia, as well as typhoid and East Coast Fever. Other diseases, such as Anthrax, tumors, and injuries can also be treated using the herbal preparations (Ibrahim *et a*l., 1998; Ruffo, 2002; Musa *et al*., 2011; Ndwigah *et al*., 2014; Wanzala *et al*., 2016). Additionally, the plant is vital in managing disorders in women, including retained afterbirth, post-delivery hemorrhage, uterine issues, as well as breast tumors (Kaingu et al., 2011; Kamau, Mbaabu, Mbaria, Gathumbi, & Kiama, 2016; Kokwaro, 2009).

Figure 2.1 Photograph of *Hydnora abyssinica*

2.5 *Uvariodendron anisatum* Verdec

2.5.1 Overall Appearance of *Uvariodendron anisatum* Verdec

Uvariodendron anisatum Verdec grows as a miniature shrub (Fig.2). It is called *Mutonga* (Kikuyu) and *Mutongu* (Meru) (Beentje, 1994; Verdcourt, 1969, 1971). The shrub is not very common. It is native to and prevalent in various parts of Central Kenya and the Eastern regions of the Country (Beentje, 1994).

Figure 2.2 Photograph of *Uvariodendron anisatum*

2.5.2 Biological activity of *Uvariodendron anisatum* Verdec

Limited research has been done on thebioactivity of *Uvariodendron anisatum* extracts are available. Misonge *et al*., (2014) reported that water extracts from *U. anisatum* roots posses oxytocic-like properties in isolated rat uterine tissue. Additionally, Mutembei *et al*. (2018) reported Uvariodendron driven antimicrobial activity against gram positive bacteria, such as *Staphylococcus aureus* as well as gram-negative bacteria for example *Escherichia coli*.

2.5.3 Phytochemistry of *Uvariodendron anisatum* Verdec

The root extract contain the compound bergenin reported by Onyancha *et al*. (2019). According to Misonge *et al*., (2014) and Mutembei *et al*., (2018), various phytochemical clusters include alkaloids, saponins, and glycosides. Other which include terpenoids and volatile oils are significant, while steroids, and phenols are additionally present in the leaf, as well as the root powder.

2.5.4 Ethnobotanical Applications of *Uvariodendron anisatum* Verdec

Root decoctions extracted from *Uvariodendron anisatum* are used to ease labour pain and are effective in relieving discomforts associated with after birth or retained placenta. At the same time, infused root extracts are used in managing infertility in men (Gachathi, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The plants used for this study were obtained from Embu County in Kenya. *Hydnora abyssinica* rhizomes were obtained from Ishiara in Mbeere-North-Sub County and *Uvariodendron anisatum* roots were obtained from Kianjiru hills in Mbeere South Subcounty.

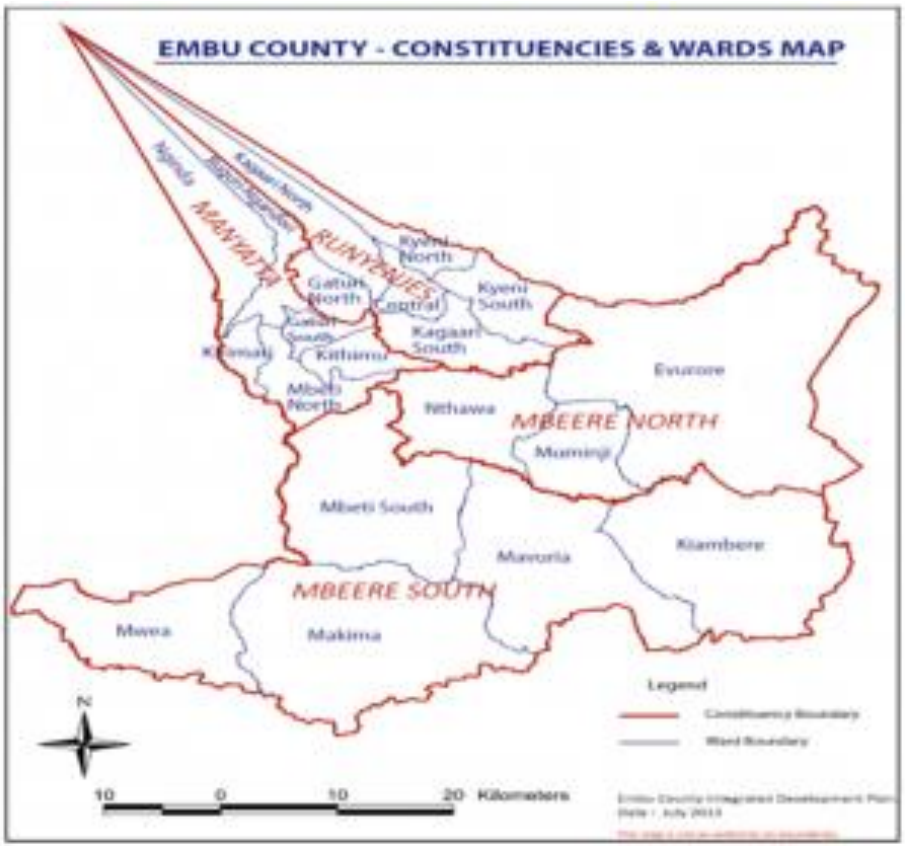


Figure 3.1 The map of Embu County showing administrative units (Embu County Government, 2019)

3.2 Study design

The study was based on laboratory experiments and standard methods as described by Kaingu *et al*. (2012) for uterine contraction activity. Acute oral toxicity studies were done following

the Organisation for Economic Co-operation and Development (OECD) guidelines No. 423 as described by Bedi & Krishan (2019). The phytochemical studies were done according to Evan's (2009).

3.3 Procedures

3.3.1 Plant Collection, Identification and Preservation

Hydnora abyssinica rhizomes and *Uvariodendron anisatum* roots were collected with the assistance of a local herbal practitioner and thereafter identification and authentication were done by a Taxonomist at the National Museums of Kenya, Botany Department. The identity of the plant specimens was further confirmed at the Department of Land Resource, Management, and Technology (LARMAT), at the University of Nairobi. The voucher specimen numbers for the plants were assigned as BMK01/17/03/2021 and BMK02/17/03/2021 for *H. abyssinica* and *U. anisatum* specimens respectively. Thereafter, voucher specimens were deposited at the East Africa Herbarium and a duplicate stored at the University of Nairobi Herbarium.

3.3.2 Preparation of extracts

3.3.2.1 Ethanol extracts

The collected plant parts, rhizome from *Hydnora abyssinica* and roots from *Uvariodendron anisatum* were cut into small pieces and spread to dry for ten days at room temperature and pressure. Thereafter, the plant parts were ground into a coarse powder by using a hammer mill. Ethanol extracts were prepared by cold maceration. Two hundred grams of each of the powdered plant materials were soaked in 250 milliliters of 80% ethanol in a 1000 ml flat-bottomed flask for 48 hours (Moriasi et al., 2020). The extracts were filtered using Whatman

paper No. 1 and thereafter reduced by employing a rotary evaporator at 35 ^{0}C followed by complete drying using an oven at 40^0 C (Evans, 2009).

3.3.2.2 Water extracts

Water extracts were prepared by freeze-drying. One hundred grams of each plant powder was boiled in 1 Liter of distilled water by using a boiling flask for one minute. The decoctions were left to cool and then filtered using Whatman paper n No. 1. The primary filtrate was centrifuged at 3000 rpm for 10 min and the supernatant filtered for a second time using sintered glass. The extracts were then lyophilized and the water extracts which were obtained were labeled and kept at 4 ^{0}C (Kaingu *et al*., 2012).

3.3.2.3 Working extract solutions

Ethanol and water extracts were reconstituted in De Jalon's physiological salt solution (Kaingu et al., 2012). De Jalon's physiological salt solution provides the needed organ bath conditions at a capacity of 40 ml in concentrates of 4.0 mg/ml, 2.0 mg/ml, 1.0 mg/ml, and 0.5 mg/ml.

3.4 Assay for uterotonic potency

3.4.1 Experimental animals

Mature female Wistar rats weighing 200 ± 5g, were used in this study. A total of 7 rats were obtained from the University of Nairobi, Department of Public Health Pharmacology and Toxicology following random selection. The rats were kept in cages in air-conditioned room at 22 ± 2 ^{0}C and 60-70% relative humidity (Wairimu et al., 2020).

Untreated wood shavings were used as bedding and changed daily. The experimental animals were exposed to approximately 12 hours of light, as well as a similar duration in the dark cycle. The rats were fed with commercial rat pellets; purchased from Unga Feeds Limited in

Kenya. The Wistar rats were allowed free access to clean water *ad libitum* (Morteza et al., 2017). All the rats were handled humanely as provided for by the Faculty of Veterinary Medicine Biosafety, Animal Use, and Ethics Committee guidelines and allowed to acclimatize for one week before the study was started.

3.4.2 Preparation of uterine tissue

Wistar rats were pretreated using Diethylstilbestrol in Arachis oil (1 mg/kg, i.p.) for 24 hours preceding the actual experiment in order to induce the estrus phase (David et al., 2019). The rats were then sacrificed humanely using phenobarbital as described by Goodies et al. (2015). Thereafter, dissection of the rats was done to access the abdominal cavity and the two horns of the uterus were carefully removed and subsequently, placed in a petri dish containing warm and aerated De Jalon's solution. All connective tissues surrounding the uterine horns were trimmed.

3.4.3 Uterotonic activity of extracts

Uterine strips of 2 cm long were cut from the uterine horns and each strip was attached vertically within the organ bath comprising of 40 ml De Jalon's which contained (0.5 g glucose, 9.0 g NaCl, 0.42 g KCl, 0.24 g $CaCl_2$, 4.5 g sucrose, 0.142 g NaH_2PO_4, as well as 2.1 g $CaHCO_3$) reconstituted in a liter of purified water. The mixture was then aerated using a blend of 5% CO_2 and 95% O_2. The organ bath temperature was maintained at $37 \pm 0.5\ ^0C$ in order to ensure tissue viability (Kaingu et al., 2012). The upper end of the uterine segment was hooked to an isometric force transducer (ML500/A, AD device) which was supplemented using Power Lab data acquisition equipment (Power Lab 8/30) (Wairimu et al., 2020). The frequency of uterine contractions (number of peaks recorded) and amplitude of contraction (in microvolts) were recorded and analyzed using Chart 5 software supported by windows. The experiments were done in triplicates, and each experiment was supplemented using a negative

control (De Jalon's solution). The study also included positive control (oxytocin 10 IU) in order to allow comparison (Kaingu et al., 2012; Wairimu et al., 2020).

3.4.4. Negative and positive control contractions

The mounted uterine strips were allowed 30 minutes to stabilize in De Jalon's solution. The contractions of the isolated uterine strips were recorded for 10 minutes. The initial contractions were taken as the negative control recordings. After 10 minutes of negative control contractions, 1.0 ml of oxytocin (10 IU) was introduced as a positive control. The isometric contractions were recorded for 10 minutes.

3.4.5 Effect of extracts on isolated Wistar rat uterine strip contractions

Fresh uterine horns were prepared as described in section 3.4.2. After the first 10 minutes of negative control contractions, 1 ml of 0.5 mg/ml of the *H. abyssinica* and *U. anisatum* extracts were introduced to the organ bath in independent experiments. Isometric contractions were recorded for 10 min followed by washing the strips three times with De Jalon's solution. A 30-minute uterine recovery time was allowed to normalize the contractions. Subsequently, the procedure was repeated with the 1.0 mg/ml, 2.0, and 4.0 mg/ml. The experiments were done in triplicates by employing unused strips for every extract concentration as well as rinsing the tissues well before each assay of the different extract concentration (Kaingu *et al*., 2012). The frequency and amplitude of uterine contractions were calculated and recorded.

3.5 Acute oral toxicity of the water extracts of *H. abyssinica* root rhizome and *U. anisatum* root

Mature female Wistar rats, aged four months and weighing 200 ± 5g, were used to evaluate acute oral toxicity of *H. abyssinica* rhizome and *U. anisatum* roots extracts. The process involved randomly selecting rats and grouping (n=3) using picric acid markings. The selected rats were kept separately for five days in polycarbonate cages in order to allow laboratory acclimatization before conducting the test. The universal protocol and instructions for severe oral toxicity given by the Organization for Economic Co-operation and Development (OECD) Guideline 423 (OECD, 2001; Bedi & Krishan, 2019) were used. Prior to treatment, the process entailed fasting experimental rats overnight and weighing. Additionally, a single dosage of the test extract was administered using gavage at 300 mg/kg and signs of toxicity were observed up to 24 hours. A repeat of 300 mg/kg was administered to another set of three experimental rats. Thereafter a higher dose of 2000 mg/kg body weight was administered to another group of three experimental rats. A repeat of 2000 mg/kg body weight dose was administered to a separate group of three experimental rats. Normal saline was used for the control group. Health parameters were observed and recorded chronologically for 2 weeks as indicated by the OECD /2001/423.

3.6 Phytochemical screening

The screening of the phytochemical groups of compounds namely, plant sterols, terpenoids (diterpenes), phenols, glycosides, fatty acids, and tannins of water and ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were done by using standard phytochemical screening procedures described by (Houghton & Raman, 1998 and Harborne,

1998) as modified by Moriasi *et al.* (2020). The tests were performed in triplicates in order to ensure the accuracy of results which were examined by visual observations.

3.6.1 Test for Alkaloids

Two tests, namely, Mayer's and Dragendorff's tests, were done in order to detect alkaloids in the extracts. Approximately 0.1 g of water and ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were mixed with 5 ml of 1 % HCL in separate test tubes respectively; each mixture was warmed and then filtered through Whatman filter paper no 1. Two drops of Mayer's reagent (mercuric potassium iodide) were added to 2 ml of water and ethanol extracts. The appearance of a cream-colored precipitate indicates the presence of alkaloids. The Dragendorff's test was carried out by adding two drops of Dragendorff's reagent (Potassium Bismuth Iodide solution) to 2 ml of the filtered water and ethanol extracts in separate test tubes. A characteristic reddish-brown precipitate was used as an indicator for the presence of Alkaloids.

3.6.2 Test for Phenolics

Approximately 100 mg of the aqueous and ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were measured and were put into separate test tubes and 10 ml of 70% ethanol were added. The mixtures were boiled in using water for five minutes. The extracts were then cooled and the extracts filtered through Whatman filter paper no 1. Five drops of 5% of ferric chloride were added into 2 ml of each respective extracts. The formation of a green precipitate indicated the presence of phenols.

3.6.3 Test for Anthraquinones

Approximately 200 mg of the aqueous and ethanolic extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* roots were added to 10 ml of ferric chloride in separate test tubes and shaken. The solutions were mixed with 5 ml Hydrochloric acid and heated on a

water-bath for 10 min. The extracts were filtered while hot using Whatman filter paper no 1. The filtrates were allowed to cool and then extracted with 10 ml of Carbon tetrachloride. The organic layer was separated and washed with 5 ml of dilute Ammonia solution. A rose-pink to cherry-red color in the Ammoniacal layer indicated the presence of anthraquinone.

3.6.4 Test for Flavonoids

Approximately 0.1 g of the aqueous and ethanolic extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were added to 10 ml ethanol (70%) in separate test tubes and warmed gently in a water bath (50°C) for 5 minutes. The extracts were filtered using Whatman filter paper no 1 and cooled. Five drops of concentrated Hydrochloric acid were added to 1 ml of Alcoholic extracts. The rapid development of a red color indicated the presence of Favonoids. The presence of Flavonoids was also confirmed by three other methods. The test sample was prepared by warming 0.1 g of the studied plant extracts in 10 ml of 70 % Ethanol and hydrolyzing with 10% Sulphuric acid. The mixture was divided into three aliquots. To the first aliquot, 1ml of dilute Ammonia solution was added, whereby a greenish-yellow colour indicates the presence of flavonoids. One milliliter (1ml) of dilute Sodium carbonate was added to the second aliquot, where the appearance of a pale-yellow colour indicates the presence of Flavonoids. Sodium hydroxide (10 %; 1 ml) was added into the third aliquot. The appearance of yellow colour is a positive test for the presence of Flavonoids.

3.6.5 Test for Tannins

Approximately 0.1 g of the aqueous and Ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were added to 10 ml of water in separate test tubes, respectively. The mixture was extracted by boiling in a water bath for five minutes. The extracts were filtered using Whatman filter paper no 1. Three drops of Ferric chloride solution were added to

2 ml of the filtrates from each extract were added three drops of Ferric chloride solution. The development of a brown-green precipitate indicated a positive test for Tannins.

3.6.6 Test for Steroids

Approximately 2 mg of the aqueous and Ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were dissolved in 1 ml of Chloroform and then shaken gently. Five drops of concentrated Sulfuric acid were added along the side of the test tube. A reddish-brown colour which was formed at the interface indicated presence of Steroids.

3.6.7 Test for Triterpenoids

In this study, 0.1g of water and Ethanol extracts from *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were dissolved in 2 ml Acetic anhydride and were heated to boiling point. The mixtures were filtered by using Whatman filter paper no 1 in order to separate test tubes. One drop of concentrated Sulphuric acid was added slowly along the sides of the test tube. An array of colour change ranging from red to blue showed the presence of Triterpenoids.

3.6.8 Test for Saponins

About 0.1g of the aqueous and Ethanol extracts of *Hydnora abyssinica* rhizome, and *Uvariodendron anisatum* root were added to 10 ml of distilled water in separate test tubes, respectively. The mixtures were boiled for 10 minutes, and they were filtered using Whatman filter paper no 1. A mixture of 3 ml distilled water and 5 ml of the filtrate were agitated vigorously for 15 seconds and left to stand for 10 minutes. The formation of a 1 cm layer of a stable honeycomb like froth which persisted for about 3 minutes was an indication of presence of saponins.

3.7 Data analysis

The mean frequency (rate) and mean amplitude (force) of uterine contractions was analyzed from the chart recordings. Mean frequency was the number of contractions over a period of ten minutes divided by the 10 and this was the number of peaks recorded. The mean amplitude or force was the mean height in microvolts (µV) of the peaks produced over the 10 minutes. By comparing the mean frequency and mean amplitude of contraction for the control and test groups, it was possible to determine whether the extract increased, reduced, or did not cause any effect in terms of mean frequency and mean amplitude of uterine contraction. Uterotonic activities was expressed as a percentage increase or decrease in mean ± standard error of the mean (SEM) relative to the controls by using the formula described by Wairimu et al. (2020).

$$\text{Percentage contractions} = \left(\frac{\text{F/At} - \text{Fc}}{\text{Fc}}\right) \text{x } 100$$

Where, F/At = $frequencyoramplitudeaftertreatment$ and Fc = $controlcontractions$

GraphPad Prism Version 8.0.1 software was used for the analysis of data. Descriptive analysis of Mean frequency and mean amplitude of uterine contractions was done by using one-way ANOVA. At the same time, levels of significance were established by using P-values at $P < 0.05$ (*); $P < 0.01$ (**) and $P < 0.001$ (***). Moreover, a post hoc Tukey's multiple comparison evaluation was conducted in order to analyze statistical differences among groups (Appendix 3 and 4).

The results from acute oral toxicity which included parameters of wellness were tabulated. The dose that killed 50% of the experimental mice (LD_{50}) were recorded and interpreted according to OECD Guideline 423 (OECD, 2001). Data for qualitative analysis of Phytochemicals was also summarized using Tables.

3.8 Ethical Consideration

Authority to conduct the study was requested from the Faculty Biosafety, Animal use and Ethics Committee (BAUEC). The permission to conduct the study was granted by BAUEC in a letter with reference number of FVMBAUEC/2021/295 (Appendix 1). Research authorization was also obtained from National Commission for Science, Technology and Innovation (NACOSTI) with a license number NACOSTI/P/21/11761 (Appendix 2).

CHAPTER FOUR

RESULTS

4.1 *In vivo* studies of isolated Wistar rat uterine strip contractility

The uterine contraction against resistance in which the length of the muscle was 2 cm (isometric contractions) were observed to increase with increased concentrations of crude extracts or standard drug (oxytocin 10 IU) under the current study. The contractions were dose dependent and contractility of the uterine strip increased up to a maximum concentration of 1 mg/ml, except for the *Uvariodendron anisatum* ethanol extract which revealed maximum contractility at 2 mg/ml.

4.1.1 Effects of *H. abyssinica* rhizome extracts on the mean force and mean frequency of Wistar rat uterine contractions

The graded concentrations of Ethanol and water extracts of *Hydnora abyssinica* rhizome revealed different effects on the mean force (amplitude) and mean frequency of the uterine contractions (Table 4.1). Percentage increase of mean force was observed with increasing concentrations of administered extracts of *H. abyssinica* rhizome on the Wistar rat uterine strips. The mean force of uterine contractility increased by 13.542 ± 0.905 at 0.5 mg/ml, and 32.547 ± 0.900 at 1 mg/ml. The mean force, dropped to 17.555 ± 0.905 at 2 mg/ml and further dropped to 8.934 ± 0.452 at 4 mg/ml of the *H. abyssinica* rhizome water extract. Figure 4.1 represents the significant difference at $p < 0.001$***, the Ethanol extract of *Hydnora abyssinica* rhizome demonstrated significantly high activity compared with that of the water extract at the corresponding graded doses and reached a maximum at 1 mg/ml after which there was a drop in the mean force of uterine contractility. The effect of oxytocin (10 IU) on

the mean force remained significantly high compared to all of the graded doses of 0.5, 1, 2 and 4 mg/ml of *Hydnora abyssinica* rhizome extracts which were studied (Figure 4.1).

Table 4.1: Effects of *H. abyssinica* extract concentrations on the mean force and frequency of uterine contractions

Extract or drug concentration	**% Mean force ± SEM**		**% Mean frequency ± SEM**	
	Water	**Ethanol**	**Water**	**Ethanol**
0.5 mg/ml	13.542 ± 0.905	44.890 ± 0.681	14.499 ± 1.026	10.134 ± 1.771
1 mg/ml	32.547 ± 0.900	70.927 ± 0.366	74.023 ± 1.026	66.822 ± 2.671
2 mg/ml	17.555 ± 0.905	65.130 ± 2.660	62.509 ± 1.026	64.969 ± 4.185
4 mg/ml	8.934 ± 0.452	55.000 ± 2.431	29.993 ± 2.052	15.115 ± 5.402
oxytocin (10 iu)	**80.206 ± 2.497**		**65.672 ± 3.735**	

n = Triplicate observations, SEM: Standard Error of the Mean

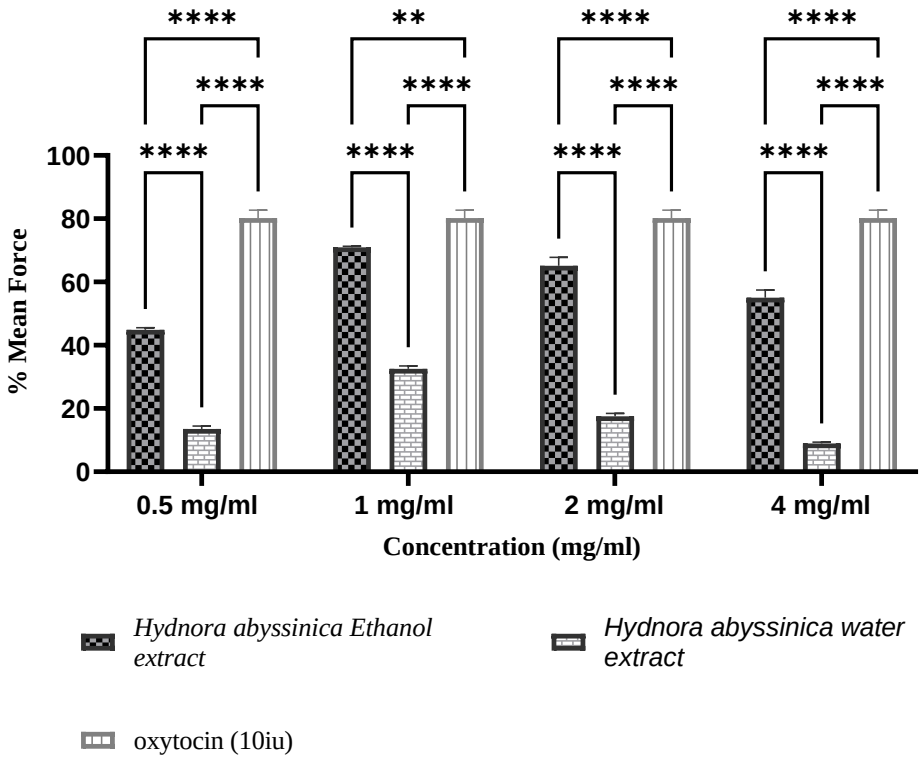


Figure 4.1: The effect of *H. abyssinica* rhizome extracts on Wistar rat uterine strip mean force contractions

Figure 4.1 shows that there was no significant difference in the effect of *Hydnora abyssinica* rhizome extracts on uterine strip frequency of contractions at $p < 0.001$*** at 0.5 mg/ml, 1 mg/ml and 2 mg/ml. The study revealed that at $p < 0.001$*** there was significant difference on the effect of oxytocin (10 IU) on the frequency of the uterine contractions at 0.5 mg/ml and 4 mg/ml. There was significant difference of the water extracts of *Hydnora abyssinica* rhizome at $p < 0.01$** at 4 mg/ml.

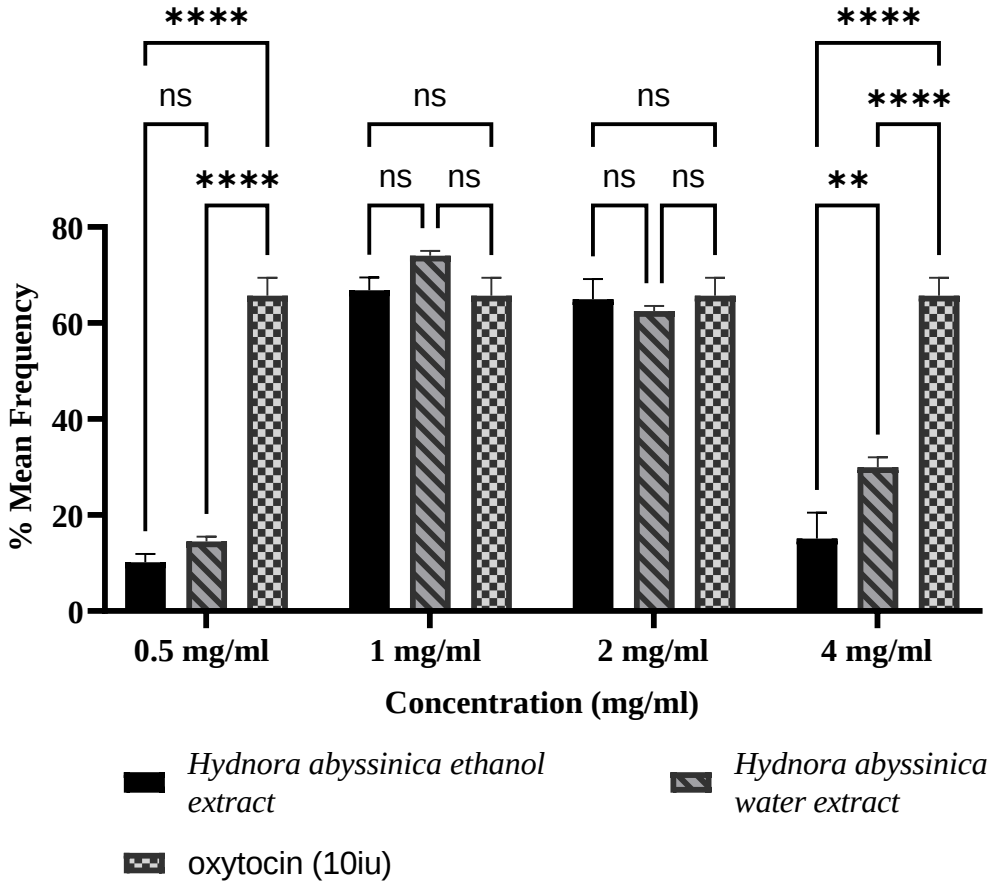


Figure 4.2: The effect of *H. abyssinica* rhizome extracts on frequency of Wistar rat uterine contractions.

4.1.2 Effects of *U. anisatum* root extracts on the mean force and frequency of Wistar rats uterine strip contractions

The water and ethanol extracts from *Uvariodendron anisatum* root revealed dose dependent effect on percentage mean force of the uterine contractions (Table 4.2). There was notable increase of percentage mean force on the contractions of the uterine strips by 27.633 ± 0.711 at 0.5 mg/ml and reached a maximum effect by 95.228 ± 2.013 at 1 mg/ml of the water extract of *Uvariodendron anisatum*. A decline of percentage mean force effect was detected from 95.228 ± 2.013 to 84.897 ± 1.631 and 66.320 ± 1.631 at 2 mg/ml and 4 mg/ml respectively for

the water extract of *Uvariodendron anisatum* root. Effects of *Uvariodendron anisatum* root extracts were also observed. The extracts increased the percentage mean force of Wistar rat uterine strip contraction. There was an increase of percentage mean force of 67.477 ± 0.826 and 69.304 ± 0.832 at concentrations of 0.5 mg/ml and 1 mg/ml respectively. Thereafter, the percentage mean force decreased such that 44.749 ± 1.065 was recorded at a concentration of 4 mg/ml. A comparison of effects of *Uvariodendron anisatum* root water extract and ethanol extract concentrations on the mean force of Wistar rats uterine strip contractions revealed a significant difference with $p < 0.001$*** at 0.5 mg/ml, 1 mg/ml and 4 mg/ml whereas there was no significant difference at a concentration 2 mg/ml (Fig 4.3).

Table 4.2: Effects of *U. anisatum* root extracts concentrations on the mean force and frequency of uterine contractions

Extract or drug concentration	**% Mean force ± SEM**		**% Mean frequency ± SEM**	
	Water	Ethanol	Water	Ethanol
0.5 mg/ml	27.633 ± 0.711	67.477 ± 0.826	39.996 ± 2.508	18.099 ± 2.970
1 mg/ml	95.228 ± 2.013	69.304 ± 0.832	78.140 ± 2.432	48.232 ± 3.851
2 mg/ml	84.897 ± 1.631	82.145 ± 0.055	34.339 ± 3.485	85.429 ± 2.970
4 mg/ml	66.320 ± 1.631	44.749 ± 1.065	22.583 ± 1.764	69.461 ± 1.485
Oxytocin (10iu)	**80.206 ± 2.497**		**65.672 ± 3.735**	

n = Triplicate observations, SEM: Standard error of the mean

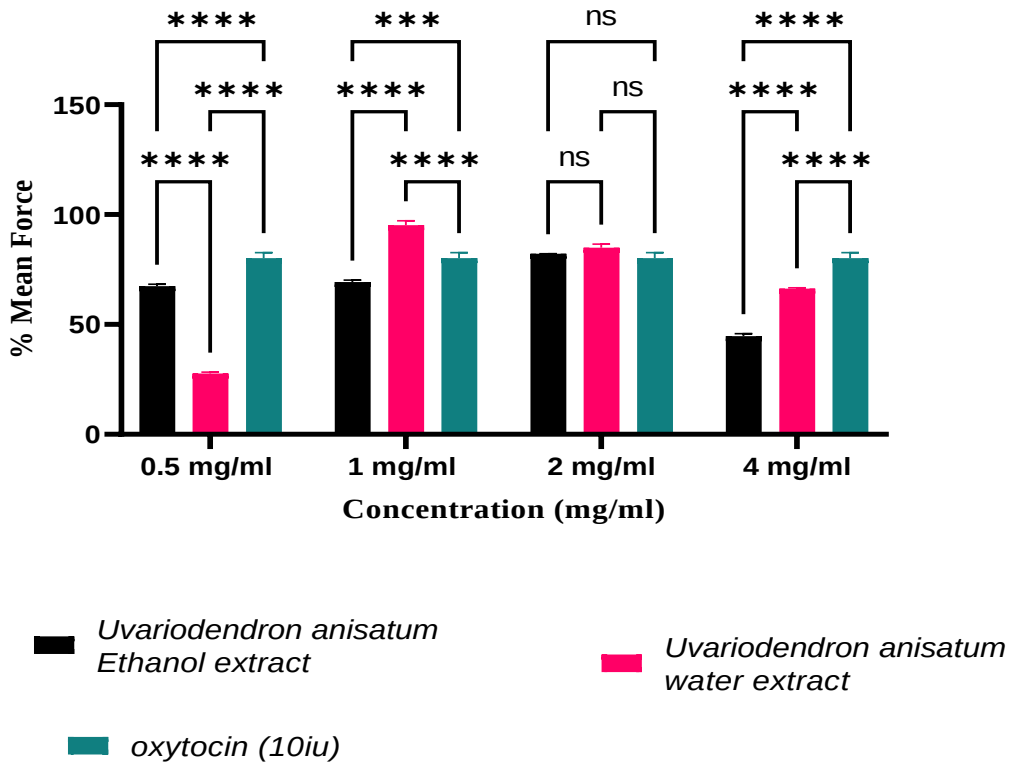


Figure 4.3: Effects of water and Ethanol extracts of *U. anisatum* root on the mean force of uterine contractions

The percentage mean frequency of uterine contractions of water extracts of *Uvariodendron anisatum* root was 39.996 ± 2.508 at a concentration of 0.5 mg/ml. At a concentration of 1 mg/ml, the percentage mean frequency increased to maximum percentage of 78.140 ± 2.432. A decline of the percentage mean frequency from 78.140 ± 2.432 to 34.339 ± 3.485 and 22.583 ± 1.764 was observed at concentrations of 2 mg/ml and 4 mg/ml respectively. The Ethanol extract, also revealed increasing percentage mean frequency of uterine contractions from 18.099 ± 2.970 to 85.429 ± 2.970 from 0.5 mg/ml to 2 mg/ml and later a decline in percentage frequency to 69.461 ± 1.485 at 4 mg/ml (Table 4.4). A comparison between percentage mean frequency of Ethanol and water extracts of *Uvariodendron anisatum* root at

different concentrations revealed significant difference $p < 0.001$*** at 0.5 mg/ml, 1 mg/ml, 2 mg/ml and 4 mg/ml (Figure 2). The Ethanol extracts revealed significantly high percentage mean frequency at concentrations of 0.5 mg/ml, 1 mg/ml and 2 mg/ml. The effect of *Uvariodendron anisatum* root water extracts on the frequency of uterine contractions was higher than that of Ethanol up to concentration of 2 mg/ml after which it dropped (Figure 4.4).

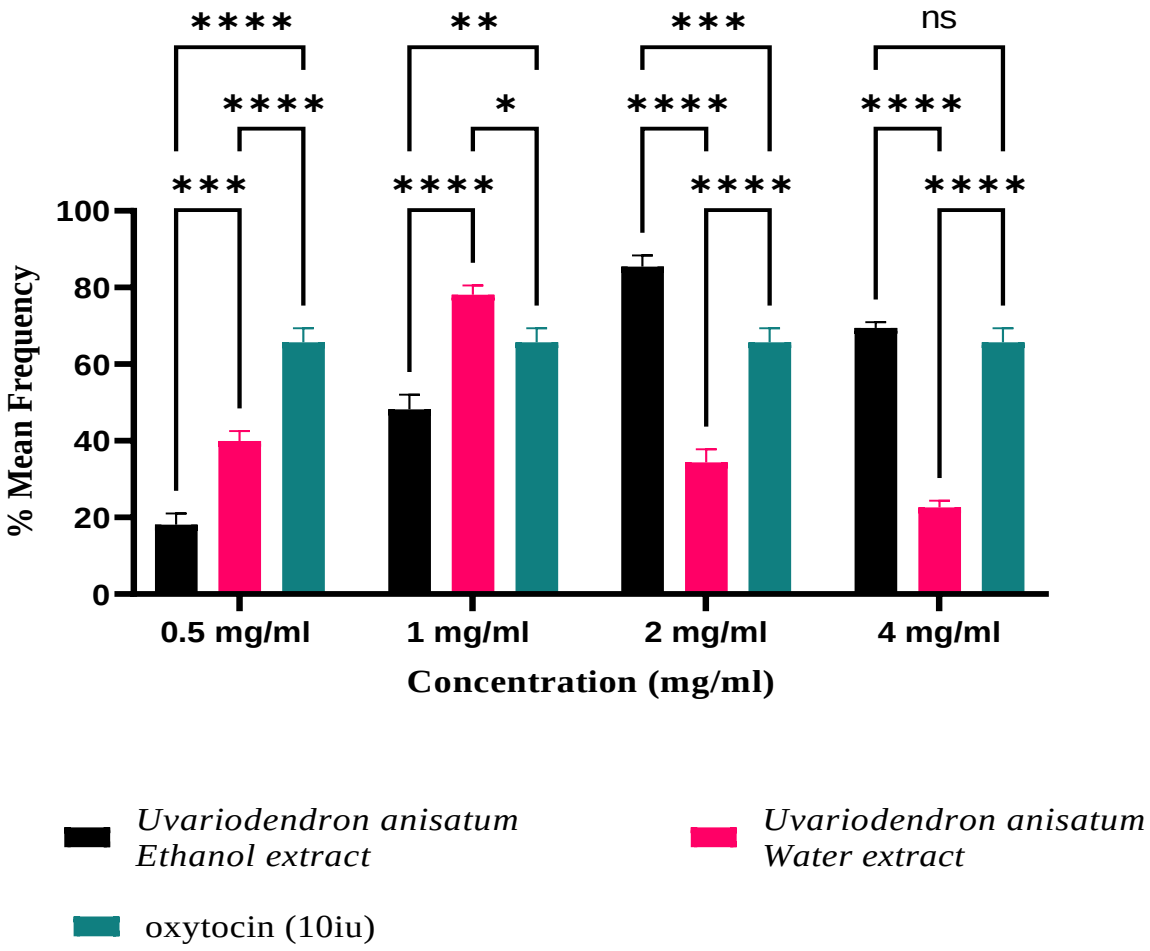


Figure 4.4: Effects of *U. anisatum* root water and ethanol extract concentrations on percentage mean frequency of uterine contractions

4.2 Toxicity of *H. abyssinica* rhizome and *U. anisatum* root extracts on female Wistar rats

The evaluation of toxic effects of a single dose of water and ethanol extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts, administered orally to Wistar female rats were assayed. The lethal dose (LD_{50}), behavioral changes and changes in blood parameters revealed that all the doses used in the current study, from 300 and 2000 mg/kg body, were non-toxic to the female Wistar rats.

4.2.1 Acute oral toxicity of *H. abyssinica* rhizome and *U. anisatum* root extracts on mortality of female Wistar rats

In the current study, all of the Wistar female rats in the treatment group survived after oral administration of single dose at 300 and a single dose of 2000 mg/kg of the water and Ethanol extracts from *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root. Therefore, there were no mortalities which were observed even after administration of the highest possible acceptable single dose of 2000 mg/kg body weight orally to all groups. In addition, the behavioral responses and general appearance of the female Wistar rats which were treated with single dose of *Hydnora abyssinica rhizome* and *Uvariodendron anisatum* root extracts were recorded in Table 4.3. None of the female Wistar rats treated with single doses of *Hydnora abyssinica rhizome* and *Uvariodendron anisatum* root extracts showed remarkable changes in clinical observations during study period.

Table 4.3: Summary of behavioural responses and general appearance of rats treated with single dose of *H. abyssinica* rhizome and *U. anisatum* root extracts in acute oral toxicity study

Observation	Control group (Physiological saline)		Experimental group			
			Single dose (300 mg/kg)		Single dose (2000 mg/kg)	
	4 hrs	24 hrs	4 hrs	24 hrs	4 hrs	24 hrs
Skin colour change	Normal	Normal	Normal	Normal	Normal	Normal
Eye colour change	Normal	Normal	Normal	Normal	Normal	Normal
General physique	No effect	No effect	No effect	No effect	No effect	No effect
Diarrhoea	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved
Coma	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved
Drowsiness	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved
Sedation	No effect	No effect	No effect	No effect	No effect	No effect
Gasping	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved
Tremor	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved	Unobserved
Salivation	Normal	Normal	Normal	Normal	Normal	Normal
Mortality	No death	No death	No death	No death	No death	No death

4.3 Phytochemical composition of *H. abyssinica* and *U. anisatum* extracts

The results of Phytochemical screening of *Hydnora abyssinica* and *Uvariodendron anisatum* powders and extracts are shown in Table 4.8 and 4.9.

4.3.1 Phytochemical composition of *H. abyssinica*

Table 4.8 Shows the results of groups of Phytochemicals which were studied on *Hydnora abyssinica* rhizome powder, Ethanol and water extracts. Alkaloids, phytosteroids, glycosides, terpenoids, diterpenoids, fatty acids proteins and phenolic compounds including flavonoids, coumarins and tannins were detected in the *H. abyssinica* rhizome powder, Ethanol and water extracts. Saponins were not observed in the *H. abyssinica* rhizome powder and Ethanol and water extracts.

Table 4.4: Phytochemical composition of *H. abyssinica* rhizome powder and extracts

Phytochemical class	Test	Powder	Ethanol extract	Water extract
Alkaloid	Drangedorff	+	+	+
Glycosides	Kedde	+	+	+
	Keller-killian	+	+	+
	Borntrager's	+	+	+
	Modified Borntrager's	+	+	+
	Legal's	+	+	+
Saponins	Foam	-	-	-
Phytosterols	Salkowski's Test	+	+	+
Triterpenes	Liebermann-Burchard	+	+	+
Diterpenes	Copper Acetate	+	+	+
Fixed oils	Filter Paper	+	+	+
Proteins	Xanthoproteic	+	+	+
Phenols	Ferric Chloride	+	+	+
Flavonoids	Alkaline	+	+	+
Tannins	Ferric chloride	+	+	+
Volatile oils	Hydro distillation	-	NT	NT

Key: (+) Present; (-) Absent; (NT) Not tested

4.3.2 Phytochemical composition of *U. anisatum*

The *U. anisatum* root powder and extracts showed the presence of alkaloids, saponins phytosteroids, glycosides, terpenoids, diterpenoids, fatty acids proteins and phenolic compounds including flavonoids (Table 4.9).

Table 4.5: Phytochemical composition of *U. anisatum* root powder and extracts

Phytochemical class	Test	Powder	Ethanol extract	Water extract
Alkaloid	Drangedorff	+	+	+
Glycosides	Kedde	+	+	+
	Keller-killian	+	+	+
	Borntrager's	+	+	+
	Modified Borntrager's	+	+	+
	Legal's	+	+	+
Saponins	Foam	+	+	+
Phytosterols	Salkowski's Test	+	+	+
Triterpenes	Liebermann-Burchard	+	+	+
Diterpenes	Copper Acetate	+	+	+
Fixed oils	Filter Paper	+	+	+
Proteins	Xanthoproteic	+	+	+
Phenols	Ferric Chloride	+	+	+
Flavonoids	Alkaline	+	+	+
Tannins	Ferric chloride	+	+	+
Essential oils	Hydro distillation	+	NT	NT

Key: (+) Present; (-) Absent**, (**NT) Not tested

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Effect of *H. abyssinica* rhizome and *U. anisatum* root extracts on Wistar rat uterine contractions

The most important finding of the current study was the scientific evidence to support the efficacy of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts in inducing uterine contractions. It was found that both the ethanol and water extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root had uterotonic activities. Uterotonic activity of *Hydnora abyssinica* rhizome extracts is reported for the first time in this study besides the existing reports of the use of *Hydnora abyssinica* water extracts in folklore medicine to treat birth related problems in women which include post-partum hemorrhage, removal of retained placenta and prevention of abortion (Bekker, 2021; Gachathi, 2007; Kaingu et al., 2011).

It was also found that both water and Ethanol extracts of *Uvariodendron anisatum* root demonstrated uterotonic activity on the uterine strips of Wistar rats. The effects of Ethanol extracts of *Uvariodendron anisatum* root on the mean force and mean frequency of the uterine tissues of Wistar rats are reported for the first time. The results of the uterotonic effects of water extract of *Uvariodendron anisatum* are consistent with the findings reported by Misonge et al. (2014). Studies of the root extracts of a plant, *Uvariodendron kirkii* (Wairimu et al., 2020). In addition, other plants of the annonaceae family are reported as useful crude drugs with uterotonic drugs (Bhardwaj et al., 2019).

5.1.2 Toxic effects of *H. abyssinica* rhizome and *U. anisatum* root extracts

The water and Ethanol extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root were classified in the range of category five (LD_{50} >2000mg/kg body weight) according to the globally harmonised classification system for chemical substances and mixtures (OECD, 2001). The absence of animal mortalities in the extract treated groups at highest dose of 2000 mg/kg body weight was interpreted as LD_{50}, which is a toxic dose which kills half of the experimental animals was more than 2000 mg/kg body weight. This indicates that single oral dose of the water and Ethanol extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root were nontoxic to Wistar rats. The acute oral toxicity findings of the Ethanol and extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root on Wistar rats were reported for the first time in this study. However, the water extracts of *Hydnora abyssinica* rhizome were reported to be nontoxic to Swiss albino rats at a dose of 1600 mg/kg body weight (Osman, 2010). Literature reports indicate that the methanol and water extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root are also nontoxic to Swiss albino mice following administration of a single oral dose at 2000 mg/kg body weight (Misonge, 2019). In addition, there were no observable clinical signs of toxicity after single dose of administration of 2000 mg/kg body weight of the water and Ethanol extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root

5.1.3 Phytochemical composition of *H. abyssinica* rhizome and *U. anisatum* root powders and extracts

The phytochemical compounds which were detected in the present study are associated with the biological activities which are used to manage various conditions in human beings. Some of the phytochemical groups of compounds are reported in this study for the first time, and

these are diterpenes and proteins in *H. abyssinica* and *Uvariodendron anisatum*. Though, other early reports are consistent with the current study where the presence of glycosides, alkaloids, phytosterol, phenols, flavonoids and triterpenes in some extract of *Hydnora abyssinica* (Onyancha et al., 2015) and *Uvariodendron anisatum* are reported (Misonge et al., 2014). The volatile oils and saponins are only reported to be present in *Uvariodendron anisatum* while they were absent in *Hydnora abyssinica.*

Some of the phytochemicals which were reported in the current study are reported to be responsible for the uterotonic activity of the plants in which they are contained. Example, a report by Gruber & Brien (2012) showed that diterpenes, glycosides, heterocyclic aldehydes, fatty acids, steroidal saponins, plant sterols and proteins have uterine stimulatory activity.

5.2 Conclusion

The following conclusions were made from the current study.

i. *Hydnora abbysinica* rhizome and *Uvariodendron anisatum* root extracts have uterotonic activity on the isolated uterine strips. This was demonstrated by the increase in percentage mean force and mean frequency of uterine strip contractions following the increase of the administered dose and compared with the standard (Oxytocin 10 IU).
ii. The extracts from the two plants, *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root are non-toxic when given orally as a single dose because there were no fatalities at doses of 2000 mg/kg body weight.
iii. *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts contain phytochemicals which are responsible for physiological activities

5.3 Recommendations

The findings of the present study show that the use of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* water preparations by traditional medicine practitioners for a long time without scientific data can be validated partially using these results.

5.3.1 Recommendations for future study

The following studies are recommended

i. Bioactivity, isolation and identification of Phytochemicals which are responsible for the uterotonic activity should be studied.
ii. Sub-chronic and chronic toxicity studies to establish the toxic effects of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* root extracts following multiple doses and a long time of exposure should be conducted.
iii. A study on mode of actions of the extracts of *Hydnora abyssinica* rhizome and *Uvariodendron anisatum* and root should also be conducted.

REFERENCES

Ahmad, K. M. S., & Ahmad, I. (2018). *Herbal Medicine: Current Trends and Future Prospects*. *Phytomedicine: Advancements in Herbal Products as Novel Drug Leads*. Elsevier Inc. https://doi.org/10.1016/B978-0-12-814619-4.00001-X

Bedi, O., & Krishan, P. (2019). Investigations on acute oral toxicity studies of purpurin by application of OECD guideline 423 in rodents.

Beentje H.J. (1994). *Kenya Trees, Shrubs and Lianas.* Nairobi: National Museums of Kenya.

Bekker, J. C. M. de. (2021). *Transcultural health care utilisation in Serengeti of Tanzania : towards applied ethnoscience in public health management*. University Leiden.

Bhardwaj, R., Pareek, S., Sagar, N. A., & Vyas, N. (2019). Bioactive Compounds of Annona. *Reference Series in Phytochemistry*.

Brendler, T., Eloff, J. N., Gurib-Fakim, A., & Phillips, L. . (2010). *African Herbal Pharmacopoeia*. *Association for African Medicinal Plants Standards* (Vol. 53).

Chotchoungchatchai, S., Marshall, A. I., Witthayapipopsakul, W., Panichkriangkrai, W., Patcharanarumol, W., & Tangcharoensathien, V. (2020). Primary health care and sustainable development goals. *Bulletin of the World Health Organization*, *98*(11), 792–800. https://doi.org/10.2471/BLT.19.245613

David, S. C., María, L. N. L., Elizabeth, A. G. H., & Beatriz, V. C. (2019). Uterotonic activity of Heimia salicifolia leaves. *Journal of Medicinal Plants Studies*, *7*(4), 98–102. Retrieved from http://www.plantsjournal.com/archives/2019/vol7issue4/PartB/7-4-7-824.pdf

Dhami, N. (2013). Trends in Pharmacognosy: A modern science of natural medicines. *Journal of Herbal Medicine*, *3*(4), 123–131. https://doi.org/10.1016/j.hermed.2013.06.001

Edhi, M. M., Aslam, H. M., Naqvi, Z., & Hashmi, H. (2013). Post partum hemorrhage: Causes and management. *BMC Research Notes*, *6*(1), 1. https://doi.org/10.1186/1756-0500-6-236

Embu County Government. County Integrated Development Plan 2018-2022 (2019). Retrieved from http://www.embu.go.ke/agriculture/

Esamai, F., Nangami, M., Tabu, J., Mwangi, A., Ayuku, D., & Were, E. (2017). A system approach to improving maternal and child health care delivery in Kenya : innovations at the community and primary care facilities (a protocol). *Reproductive Health*, 1–18. https://doi.org/10.1186/s12978-017-0358-6

Evans, W. C. (2009). *Trease and Evans Pharmacognosy* (Sixteent E). London: Saunders/Elsevier.

Fukami, T., Koga, H., Goto, M., Ando, M., Matsuoka, S., Tohyama, A., … Tsujioka, H. (2019). Incidence and risk factors for postpartum hemorrhage among transvaginal deliveries at a tertiary perinatal medical facility in Japan. *PLoS ONE*, *14*(1), 1–8. https://doi.org/10.1371/journal.pone.0208873

Gachathi M.N.F. (2007). *Kikuyu botanical dictionary - A guide to plant names and cultural values* (revised 2n). Kenya: Tropical botany.

Gewali, B. M. (2008). Aspects of Traditional Medicine Aspects of Traditional Medicine in Nepal. *Institute of Natural Medicine*, 175.

Gitobu, C. M., Gichangi, P. B., & Mwanda, W. O. (2018). The effect of Kenya's free maternal health care policy on the utilization of health facility delivery services and maternal and neonatal mortality in public health facilities. *BMC Pregnancy and Childbirth*, *18*(1), 1–11. https://doi.org/10.1186/s12884-018-1708-2

Goodies, M. E., Oghenesuvwe, E. E., & Ejiroghene, A. (2015). Effects of inhaled anaesthetic agents (Chloroform and Diethyl ether) on fasting blood glucose and haematological parameters in Wistar rats. *Sky Journal of Biochemistry Research*, *4*(2), 013–015.

Gruber, C. W., & Brien, M. O. (2012). Uterotonic Plants and their Bioactive Constituents, *77*(3), 207–220. https://doi.org/10.1055/s-0030-1250317.Uterotonic

Hajj, M. El, & Holst, L. (2020). Herbal Medicine Use During Pregnancy : A Review of the Literature With a Special Focus on Sub-Saharan Africa, *11*(June), 1–13. https://doi.org/10.3389/fphar.2020.00866

Harborne, J. B. (1998). Phytochemical Methods A Guide To Modern Tecniques Of Plant Analysis, Third Edition. *Chapman & Hall*, 58. https://doi.org/10.1017/CBO9781107415324.004

Houghton, P. J., & Raman, A. (1998). *Laboratory Handbook for the Fractionation of Natural Extracts*. *Laboratory Handbook for the Fractionation of Natural Extracts*. https://doi.org/10.1007/978-1-4615-5809-5

Ibrahim, F., Ibrahim, B., Bayreuth, U., & Bayreuth, D. (1998). The Maasai Herbalists in Arusha Town , Tanzania. *GeoJournal*, *46*, 141–154.

International Council for Science. (2015). *Review of the Sustainable Development Goals: The Science Perspective.* Paris.

Jeruto, P., Mutai, C., Catherine, L., Ouma, G., & Otieno, D. (2008). "An ethnobotanical study of medicinal plants used by the Nandi people in Kenya." *Journal of Ethno-Pharmacology*, *116*(1), 370–376.

Kaingu, C. K., Oduma, J. A., & Kanui, T. (2012). Preliminary investigation of contractile activity of Ricinus communis and Euclea divinorum extracts on isolated rabbit uterine strips. *Journal of Ethnopharmacology*, *142*(2), 496–502.

https://doi.org/10.1016/j.jep.2012.05.026

Kaingu, C. K., Oduma, J. A., & Kanui, T. I. (2011). Practices of Traditional Birth Attendants in Machakos District , Kenya. *Journal of Ethnopharmacology*, *137*(1), 495–502. https://doi.org/10.1016/j.jep.2011.05.044

Kaingu, C. K., Oduma, J. A., Mbaria, J. M., & Kiama, S. G. (2017). Effects of Croton menyharthii and Uvariodendron kirkii extracts on ovarian corpora lutea and reproductive hormones. *Discovery Phytomedicine*, *4*(2), 21. https://doi.org/10.15562/phytomedicine.2017.41

Kamau, L. N., Mbaabu, P. M., Mbaria, J. M., Gathumbi, P. K., & Kiama, S. G. (2016). Ethnobotanical Survey and threats to medicinal plants traditionally used for the management of human diseases in Nyeri County , Kenya. *Tang (Humanitas Medicine)*, *6*(3), 1–15.

Kokwaro, J. O. (2009). *Medicinal plants of east Africa. Nairobi: University Press*. Kenya: East Africa Literature Bureau.

Krymow, V. (2012). *Plants of the Bible. Accessed online at http://www.americancatholic.org/Newsletters/SFS/an0304. on 13th October 2020*.

Kupittayanant, S., Munglue, P., Lijuan, W., Promprom, W., Budhaklala, N., & Wray, S. (2014). Finding new agents in medicinal plants to act on the myometrium. *Experimental Physiology*, *99*(3), 530–537. https://doi.org/10.1113/expphysiol.2013.072884

Likis, F., Nila, A., Morgans, K., Hartmann, K., Young, J., Carlson-Bremer, D., … Andrews, J. (2015). Management of Postpartum Hemorrhage Comparative Effectiveness Review Management of Postpartum Hemorrhage. *Agency for Healthcare Research and Quality*, (151).

Maundu, M. (1999). *Traditional food plants of Kenya. Kenya*. Nairobi, Kenya: National Museums of Kenya.

Misonge, J. O., Ogeto, J. O., Sengera, G. O., Mwalukumbi, J. M., Mwaura, A. M., & Juma, S. D. (2014). Evaluation of phytochemistry and uterotonic activity of root aqueous extract of Uvariodendron anisatum verdec . used in childbirth in Eastern / Central Kenya . *IOSR Journal Of Pharmacy*, *4*(12), 48–53.

Misonge J O. (2019). *Evaluation of Antibreast and Antiprostate Cancer Activities of Selected Medicinal Plants From Some Parts of Kenya*. PhD Thesis, Kenyatta University.

Moriasi, G. A., Ireri, A. M., & Ngugi, M. P. (2020). In Vivo Cognitive-Enhancing , Ex Vivo Malondialdehyde- Lowering Activities and Phytochemical Profiles of Aqueous and Methanolic Stem Bark Extracts of Piliostigma thonningii (Schum .). *International Journal of Alzheimer's Disease*, *2020*, 1–15.

Moriasi, G., Ireri, A., & Ngugi, M. (2020). Cognitive-Enhancing, Ex Vivo Antilipid Peroxidation and Qualitative Phytochemical Evaluation of the Aqueous and Methanolic Stem Bark Extracts of Lonchocarpus eriocalyx (Harms.) . *Biochemistry Research International*, *2020*, 1–16. https://doi.org/10.1155/2020/8819045

Morris, J. L., Stenson, A., Theiss-Nyland, K., Coelius, R., Tudor, C., Cuomu, M., & Miller, S. (2011). Preventing Postpartum Hemorrhage: Comparing ZB11, a Traditional Tibetan Medicine, to Misoprostol. *International Journal of Childbirth*, *1*(3), 159–170. https://doi.org/10.1891/2156-5287.1.3.159

Morteza, I. B., Musa, G. S., Katayoun, S., Morteza, J., Hanieh, N., & Mahdi, Z. K. (2017). The Effect of Hydro-Alcoholic Extract of Rosa damascena on Uterine Constriction of Virgin Rats. *Middle East Journal of Rehabilitation and Health*, *In Press*(In Press). https://doi.org/10.5812/mejrh.13089

Mudonhi, N., & Nunu, W. N. (2021). Traditional medicine utilisation and maternal complications during antenatal care among women in Bulilima , Plumtree , Zimbabwe. *Maternal Health, Neonatology, and Perinatology*, 1–9.

Mudonhi, N., Nunu, W. N., & Sibanda, N. (2021). Exploring traditional medicine utilisation during antenatal care among women in Bulilima District of Plumtree in Zimbabwe. *Scientific Reports*, (0123456789), 1–9. https://doi.org/10.1038/s41598-021-86282-3

Musa, M. S., Abdelrasool, F. E., Elsheikh, E. A., Ahmed, L. A. M. N., Mahmoud, A. L. E., & Yagi, S. M. (2011). Ethnobotanical study of medicinal plants in the Blue Nile State , South-eastern Sudan. *Journal of Medicinal Plants Research*, *5*(17), 4287–4297.

Ndwigah, S. N., Amugune, B. K., Thoithi, G. N., Mwangi, J. W., Mugo, H. N., & Kibwage, I. O. (2014). Antibacterial and Antifungal Activity of Dombeya torrida (J . F . Gmel) and Hydnora abyssinica (A . *Afr. J. Pharmacol. Ther.*, *3*(1), 14–18.

OECD. (2001). *OECD Guideline for testing of chemicals: acute Oral Toxicity – Acute Toxic Class Method. No. 423*.

Onyancha, J., Cherongis, C., Nzivo, J., Muriithi, G., Njuguna, D., & Mwalukumbi, J. (2015). Phytochemical screening and evaluation of antioxidant activity of methanolic extract of Kenyan Hydnora abyssinica A . Braun. *Journal of Innovations in Pharmaceuticals and Biological Sciences*, *2*(1), 1-.

Onyancha, J., Gikonyo, N., Wachira, S., & Gicheru, M. (2019). Isolation and antiproliferative characterisation of bergenin from root extract of Uvariodendron. *Drug Discovery*, *13*, 55–78.

Onyancha, M. J., Gikonyo, K. N., Wachiram, W. S., & Gicheru, M. M. (2019). An ethnobotanical survey of plants used for the treatment and management of cancer in Embu County, Kenya. *Journal of Medicinal Plants Studies*, *7*(4), 39–46. Retrieved

from http://web.b.ebscohost.com.libproxy.lib.unc.edu/ehost/detail/detail?vid=11&sid=fe6e3aff-b35a-45dc-9a00-024257d75e36%40pdc-v-sessmgr06&bdata=JnNpdGU9ZWhvc3QtbGl2ZQ%3D%3D#AN=20153114436&db=lhh

Osman, H. M. Y. (2010). *Anti-diarrhoeal Activity of Hydnora abyssinica Aqueous Root Extract in Rats*. University of Khartoum.

Park, Y. L., & Canaway, R. (2019). Integrating traditional and complementary medicine with national healthcare systems for universal health coverage in asia and the Western Pacific. *Health Systems and Reform*, *5*(1), 24–31. https://doi.org/10.1080/23288604.2018.1539058

Riang'a, R. M., Nangulu, A. K., & Broerse, J. E. W. (2018). Perceived causes of adverse pregnancy outcomes and remedies adopted by Kalenjin women in rural Kenya. *BMC Pregnancy and Childbirth*, *18*(1), 1–16. https://doi.org/10.1186/s12884-018-2041-5

Ruffo, C. K. (2002). *Edible Wild Plants of Tanzania*. (A. K. Lindqvist, Ed.). Nairobi, Kenya: Regional Land Management Unit/Sida.

Saad, B., Zaid, H., Shanak, S., & Kadan, S. (2017). Introduction to Medicinal Plant Safety and Efficacy. In *Anti-diabetes and Anti-obesity Medicinal Plants and Phytochemicals* (pp. 21–56). Springer International Publishing AG. https://doi.org/10.1007/978-3-319-54102-0

Saadabi, A. M. A., & Ayoub, S. M. H. (2009). Comparative bioactivity of Hydnora abyssinica A . Braun against different groups of fungi and bacteria. *Journal of Medicinal Plants Research*, *3*(December 2008), 262–265.

Sarmiento, I., Zuluaga, G., & Andersson, N. (2016). Traditional medicine used in childbirth and for childhood diarrhoea in Nigeria's Cross River State: Interviews with traditional practitioners and a statewide cross-sectional study. *BMJ Open*, *6*(4), 1–8. https://doi.org/10.1136/bmjopen-2015-010417

Shewamene, Z., Dune, T., & Smith, C. A. (2017). The use of traditional medicine in maternity care among African women in Africa and the diaspora: A systematic review. *BMC Complementary and Alternative Medicine*, *17*(1). https://doi.org/10.1186/s12906-017-1886-x

Tripathi, V., Stanton, C., & Anderson, F. W. J. (2013). Traditional preparations used as uterotonics in Sub-Saharan Africa and their pharmacologic effects. *International Journal of Gynecology and Obstetrics*, *120*(1), 16–22. https://doi.org/10.1016/j.ijgo.2012.06.020

Tsitsi, P., & Precious, S. (2016). The African Pitocin - a midwife's dilemma: the perception of women on the use of herbs in pregnancy and labour in Zimbabwe, Gweru. *PanAfrican Medical Journal*, *8688*, 1–7. https://doi.org/10.11604/pamj.2016.25.9.7876

Verdcourt, B. (1969). The genus Uvariodendron (Engl. & Diels) R. E. Fries (Annonaceae) in East Africa. *Kew Bulletin*, *23*(3), 511–521.

Verdcourt, B. (1971). *Flora of Tropical East Africa.* Kew: Bull.

Wairimu, E., Charles, K., Maina, I., Kaingu, C. K., & Wafula, D. K. (2020). Evaluation of oxytocin like effects of Uvariodendron kirkii (Verdec .) extracts on isolated uterine strips of Wistar rats. *Humanitas Medicine*, *10*(1), 1–8.

Waleed S. Koko, Elbadri E. Osman, M. G. (2009). Antioxidant and antiglycation potential of some Sudanese medicinal plants and their isolated compounds. *Boletín Latinoamericano y Del Caribe de Plantas Medicinales y Aromáticas*, *8*(5), 402–411.

Waleed, S., Mohamed, A., Rosa, R., Mohamed, G., & Muhammad, I. (2015). Immunosuppressive phenolic compounds from Hydnora abyssinica A . Braun. *BMC Complementary and Alternative Medicine*, *15*(400), 1–9. https://doi.org/10.1186/s12906-015-0931-x

Wanzala, W., Syombua, S. M., & Alwala, J. O. (2016). A survey of the applications and use of ethnomedicinal plants and plant products for healthcare from the Ukambani region in Eastern Kenya. *Indian Journal of Ethnophytopharmaceuticals*, *2*(2), 6–58.

World Health Organisation (WHO). (2015). *Trends in maternal mortality 1990 to 2015. Estimates developed by WHO, UNICEF, UNFPA and The World Bank.* Retrieved from http://datatopics.worldbank.org/hnp/files/Trends in Maternal Mortality 1990 to 2015 full report.PDF

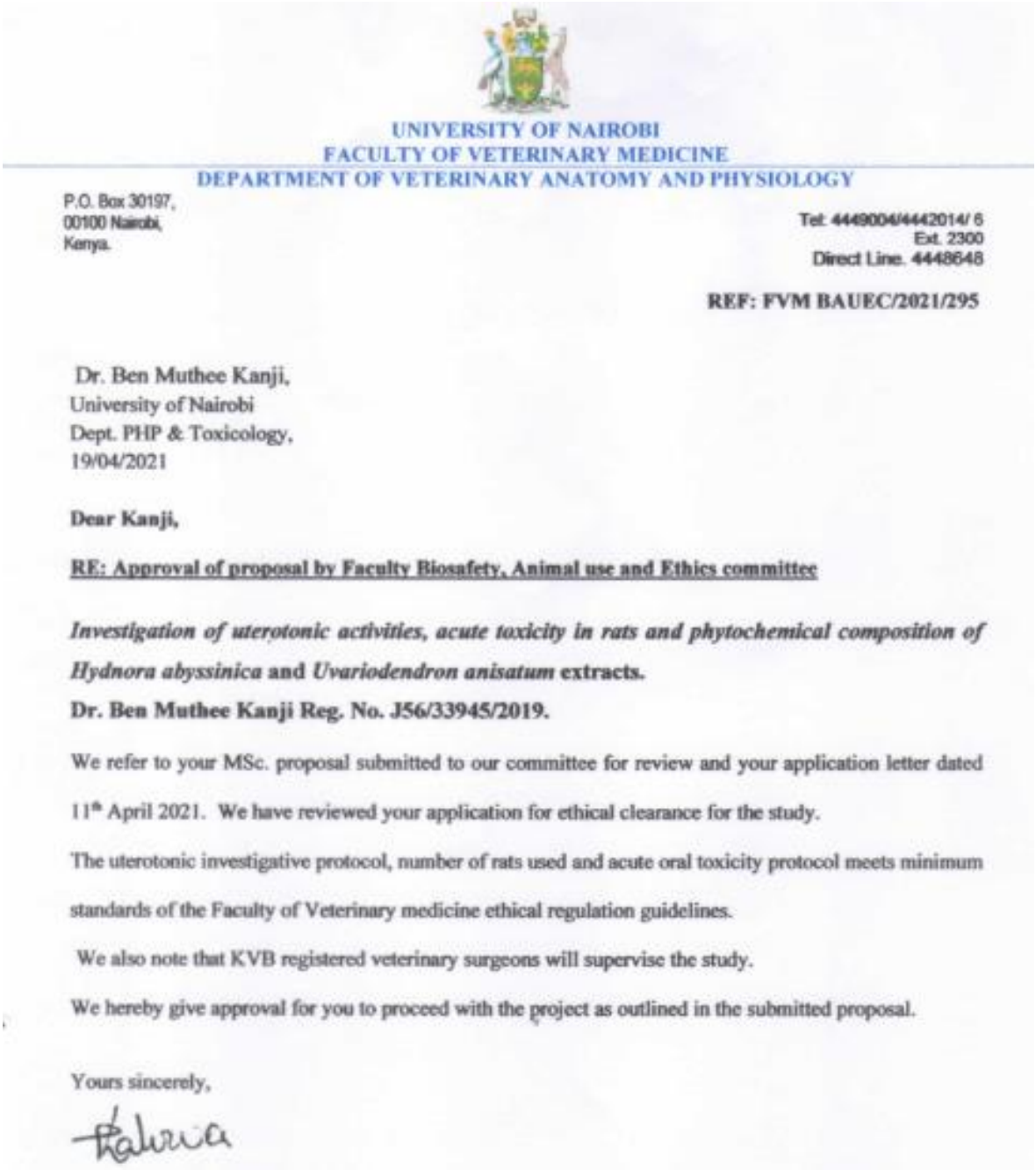
World Health Organization. (2019). *WHO Global report on traditional and complementary medicine 2019. World Health Organization*. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/312342/9789241515436-eng.pdf?ua=1

Yagi, S., Chrétien F., Duval R.E., Fontanay S., Maldini M.Piacente S., Henry M., Chapleur Y., & Laurain-Mattar, D. (2012). Antibacterial activity , cytotoxicity and chemical constituents of Hydnora johannis roots. *South African Health Review2*, *78*, 228–234. https://doi.org/10.1016/j.sajb.2011.09.010

Zamawe, C., King, C., Jennings, H. M., & Fottrell, E. (2018). Associations between the use of herbal medicines and adverse pregnancy outcomes in rural Malawi: A secondary analysis of randomised controlled trial data. *BMC Complementary and Alternative Medicine*, *18*(1), 1–8. https://doi.org/10.1186/s12906-018-2203-z

APPENDICES

APPENDIX 1: ETHICAL APPROVAL OF THE STUDY BY THE FACULTY OF VETERINARY MEDICINE BIOSAFETY, ANIMAL USE AND ETHICS COMMITTEE



UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

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REF: FVM BAUEC/2021/295

Dr. Ben Muthee Kanji,
University of Nairobi
Dept. PHP & Toxicology,
19/04/2021

Dear Kanji,

RE: Approval of proposal by Faculty Biosafety, Animal use and Ethics committee

***Investigation of uterotonic activities, acute toxicity in rats and phytochemical composition of Hydnora abyssinica* and *Uvariodendron anisatum* extracts.**
Dr. Ben Muthee Kanji Reg. No. J56/33945/2019.

We refer to your MSc. proposal submitted to our committee for review and your application letter dated 11th April 2021. We have reviewed your application for ethical clearance for the study.

The uterotonic investigative protocol, number of rats used and acute oral toxicity protocol meets minimum standards of the Faculty of Veterinary medicine ethical regulation guidelines.

We also note that KVB registered veterinary surgeons will supervise the study.

We hereby give approval for you to proceed with the project as outlined in the submitted proposal.

Yours sincerely,

APPENDIX 2: NACOSTI RESEARCH LICENCE

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **683790**

Date of Issue: **22/July/2021**

RESEARCH LICENSE

This is to Certify that Dr.. BENSON MUTHEE KANJI of University of Nairobi, has been licensed to conduct research in Embu on the topic: Investigation of Uterotonic Activities, Acute Toxicity in Rats and Phytochemical Composition of Hydnora Abyssinica and Uvariodendron Anisatum Extracts for the period ending : 22/July/2022.

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APPENDIX 3: DATA ANALYSIS OUTPUTS OF ONE-WAY ANOVA OF EFFECT OF EXTRACTS ON MEAN FORCE OF UTERINE CONTRACTIONS

MEAN FORCE

Tukey's multiple comparisons test	Summary	Adjusted P Value	
0.5 mg/ml			
Group A vs. Group B	****	<0.0001	KEY
Group A vs. Group C	****	<0.0001	A=HAE
Group A vs. Group D	****	<0.0001	B=HAW
Group B vs. Group C	****	<0.0001	C=UAE
Group B vs. Group D	****	<0.0001	D=UAW
Group C vs. Group D	****	<0.0001	
01 mg/ml			
Group A vs. Group B	****	<0.0001	
Group A vs. Group C	ns	0.8893	
Group A vs. Group D	****	<0.0001	
Group B vs. Group C	****	<0.0001	
Group B vs. Group D	****	<0.0001	
Group C vs. Group D	****	<0.0001	
2 mg/ml			
Group A vs. Group B	****	<0.0001	

Group A vs. Group C	****	<0.0001
Group A vs. Group D	****	<0.0001
Group B vs. Group C	****	<0.0001
Group B vs. Group D	****	<0.0001
Group C vs. Group D	ns	0.619
4 mg/ml		
Group A vs. Group B	****	<0.0001
Group A vs. Group C	***	0.0003
Group A vs. Group D	****	<0.0001
Group B vs. Group C	****	<0.0001
Group B vs. Group D	****	<0.0001
Group C vs. Group D	****	<0.0001
oxytocin (10iu)		
Group A vs. Group B	ns	>0.9999
Group A vs. Group C	ns	>0.9999
Group A vs. Group D	ns	>0.9999
Group B vs. Group C	ns	>0.9999
Group B vs. Group D	ns	>0.9999
Group C vs. Group D	ns	>0.9999

APPENDIX 4: DATA ANALYSIS OUTPUTS OF ONE-WAY ANOVA OF EFFECT OF EXTRACTS ON MEAN FREQUENCY OF UTERINE CONTRACTIONS

Mean frequency

Tukey's multiple comparisons test	Summary	Adjusted P Value	
0.5 mg/ml			
Group A vs. Group B	ns	0.7374	KEY
Group A vs. Group C	ns	0.2589	A=HAE
Group A vs. Group D	****	< 0.0001	B=HAW
Group B vs. Group C	ns	0.8335	C=UAE
Group B vs. Group D	****	< 0.0001	D=UAW
Group C vs. Group D	****	< 0.0001	
1 mg/ml			
Group A vs. Group B	ns	0.344	
Group A vs. Group C	***	0.0005	
Group A vs. Group D	ns	0.0535	
Group B vs. Group C	****	< 0.0001	
Group B vs. Group D	ns	0.7703	
Group C vs. Group D	****	< 0.0001	
2 mg/ml			
Group A vs. Group B	ns	0.9387	
Group A vs. Group C	***	0.0001	
Group A vs. Group D	****	< 0.0001	

Group B vs. Group C	****	< 0.0001
Group B vs. Group D	****	< 0.0001
Group C vs. Group D	****	< 0.0001

4 mg/ml

Group A vs. Group B	**	0.0064
Group A vs. Group C	****	< 0.0001
Group A vs. Group D	ns	0.3124
Group B vs. Group C	****	< 0.0001
Group B vs. Group D	ns	0.3192
Group C vs. Group D	****	< 0.0001

Oxytocin (10iu)

Group A vs. Group B	ns	> 0.9999
Group A vs. Group C	ns	> 0.9999
Group A vs. Group D	ns	> 0.9999
Group B vs. Group C	ns	> 0.9999
Group B vs. Group D	ns	> 0.9999
Group C vs. Group D	ns	> 0.9999

Key: A**=** *Hydnora abyssinica* rhizome ethanol extract; B **=** *Hydnora abyssinica* rhizome water extract**;** C = *Uvariodendron anisatum* root ethanol extract; D = *Uvariodendron anisatum* root ethanol extract**;** ns = not significant; * = significant at P < 0.05; ** = significant at P < 0.01 and *** = significant at P < 0.001; vs = versus

APPENDIX 5: SUMMARY OF PLAGIARISM REPORT

31/8/22

Investigation of Uterotonic activities, Acute Toxicity in rats and Phytochemical composition of Hydnora abyssinica and Uvariodendron anisatum extracts

ORIGINALITY REPORT

15%	12%	6%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Catherine Kaluwa Kaingu, Jemimah Achieng Oduma, Titus Kanui. "Preliminary investigation of contractile activity of Ricinus communis and Euclea divinorum extracts on isolated rabbit uterine strips", Journal of Ethnopharmacology, 2012 Publication	1%
2	aquadocs.org Internet Source	1%
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Exclude quotes On Exclude matches < 1 words

APPENDIX 6: ABSTRACT OF A PAPER PUBLISHED FROM PART OF THE PRESENT WORK

Hindawi
Evidence-Based Complementary and Alternative Medicine
Volume 2022, Article ID 7393537, 12 pages
https://doi.org/10.1155/2022/7393537

Research Article

Evaluation of Uterotonic Activity, Acute Oral Toxicity, and Phytochemical Composition of *Uvariodendron anisatum* Verdc. Root Extracts

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Over 80% of cultural societies in low-income countries use plant preparations in traditional medicine with unknown potency and safety profiles. *Uvariodendron anisatum* root extracts are used by some Kenyan herbalists. However, the claims of the plant to remove retained placenta during birth have remained uninvestigated. Therefore, the current study evaluated its uterotonic activities. Acute toxicity in Wistar rats and the phytochemical composition of the plant were also studied. The plant was collected from Embu County in Kenya. The water and ethanol extracts were prepared by maceration. Uterine strips were isolated from primed mature female Wistar rats and used to study the uterotonic activities of the extracts. De Jalon's solution and oxytocin were used as negative and positive controls, respectively. Acute oral toxicity studies were done following the OECD 423 guideline and phytochemical screening were based on standard phytochemical procedures. The study met all the approval requirements before commencement. Data obtained from the uterotonic activity were analysed by using GraphPad Prism Version 8.0.1 software and expressed as a percentage increase or decrease of mean as mean ± SEM relative to the controls. The findings of acute oral toxicity were expressed using LD_{50}. Additionally, the phytochemical components of the *U. anisatum* were tabulated. The uterotonic effect of *Uvariodendron anisatum* root water extract was higher than that of ethanol extract. A single dose of the *Uvariodendron anisatum* root water extract at 2000 mg/kg did not cause mortality in the tested Wistar rats. Besides, there were no changes in hematological and biochemical parameters. The extracts did not reveal changes in the gross morphology of the liver, kidney, heart, and lung of the tested Wistar rats. However, the histopathological studies of *Uvariodendron anisatum* root water extracts exhibited toxicity in the liver, kidney, and lung tissues of Wistar rats at a concentration of 2000 mg/kg. Alkaloids, glycosides, saponins, phytosterols, terpenes, proteins, phenols, and oils were recorded in *Uvariodendron anisatum*. The findings from this study provided scientific evidence which is useful in validating the use of *Uvariodendron anisatum* extracts in the stimulation of the uterus during birth.