COMPARISON OF PHYTOCHEMICAL AND BIOACTIVITY OF HERBAL MEDICINES USED FOR INDUCTION OF LABOUR FROM KAGERA AND NJOMBE TANZANIA

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Comparison of Phytochemicals and Bioactivity of Herbal Medicines used for Induction of Labour from Kagera and Njombe Tanzania

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation entitled: **"*Comparison of Phytochemicals and Bioactivity of Herbal Medicines used for Induction of Labour in Kagera and Njombe in Tanzania,*"** in (Partial) fulfillment of the requirement for the degree of Masters of Science (Biochemistry) of Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT

I, **Mlekwa Nour Mgawe,** 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.

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ABREVIATIONS

AA	Arachidonic Acid
ACh	Acetylcholine
AOC	Agonist Operated Calcium Channel
DAG	Diacylglycerol
ECM	Extracellular matrix
ERB	Ethical Review Board
ERC	Ethical Review Committee
ET	Endotherine
ITM	Institute of Traditional Medicine
MLCK	Myosin light chain kinase
MUHAS	Muhimbili University of Health and Allied Sciences
OTR	Oxytocin Receptor
PG	Prostaglandin
PPH	Postpartum hemorrhage
PSS	Physiological saline/salt solution
SR	Sarcoplasmic Reticulum
TBAs	Traditional Birth Attendants
TPDF	Tanzania Peoples Defense Force
UNDP	United Nations Development Programme
VOC	Voltage Operated Calcium Channel
WHO	World Health Organization

DEFINITION OF TERMS

Phytochemicals are natural chemical compounds present in plants which possess biological activities beneficial to human health

Bioactivity is the characteristics of materials which introduce in the living system to interact with that system such as tissue response or to initiate specific response of the living tissue after the introduction of that materials

Crude extract is the concentrated of substance obtained from a mixture or materials such as plants cell, culture or body tissues by chemicals or physical means which includes digestions (using enzymes), distillation, solvents actions or mechanical separation

Fetal distress is the occurrence of the indication which shows that the fetal is not in good condition and includes but not limited to fetal movement decreases and meconium in the amniotic fluid for non-breach presentation.

Induction of labour is the treatment which stimulate the uterine contraction before spontaneous onset of labour. It can be induced by pharmaceutical or non-pharmaceutical

Labour argumentation is the treatment which increase the strength and frequency after the onset of labour

Labour is the process which involve the expulsion of the baby, placenta and all uterine content

Local herbs are plants which are locally available in the community used for therapeutic purposes or as the spices in the food.

Postpartum hemorrhage is the loss of 500 mls of blood or above in the first 24 hours post-delivery.

Receptor is the special structure on the cell membrane which binds specific molecules (ligand) for cellular communication/signal transduction.

Uterine contraction is the contraction of smooth muscles of the uterus which occurs during labour and menstruation as well

Phytochemical screening is the process of determining the types/ groups of phytochemicals present in the crude extract of the plant extracts excluding

Extraction is the process of obtaining crude extract from the plant materials by using 80% aqueous - ethanol.

Tissue bath technique is the process which used to determine uterine contraction by using strip of the rat uterine myometrium which placed in the physiological saline (PSS) and then exposed to the test crude extract.

ABSTRACT

Background: The use of medicinal plants in Africa and in many developing countries has led to increased interest by the World Health Organization (WHO) to promote extensive studies on medicinal plants (local herbs). Many studies conducted in Africa have shown that **Traditional Birth Attendants** use the local herbs for management of labour and related complications such as; prolonged labour, retained placenta and post-partum hemorrhage.

Broad objective: To compare phytochemical constituents and bioactivity of herbal medicines used for induction of labour from Kagera and Njombe Tanzania.

Materials and methods: This was an experimental study design where the uterotonic effects of extracts of *Comelina africana* and *Biophytum helenae* from two regions of Tanzania namely; Njombe and Kagera were assessed. Oxytocin was used as the positive control and physiological saline solution (PSS) was used as negative control. Dried plant materials were macerated with 80% ethanol in water at room temperature to get crude extracts. Uterotonic effects of crude extracts were determined as the average effect of uterine contraction using tissue bath technique.

Results: from Kagera and that from Njombe with *p= 0.7 for C. africana* and *p= 0.9 for B. helenae* The phytochemical screening showed presence of; saponins and cardiac glycosides which play a role in uterine contraction. Determination of the uterotonic effect showed that *C. africana* from Kagera induced contraction with minimum effect of 27.2% and maximum effect of 95. 8% while *C. africana* from Njombe induced 31.3% minimum and 82.5% maximum effect. Extracts of *B. helenae* from Kagera induced 28.1% minimum effect and 97.3% and *B. helenae* Njombe with 29.2% and 94.8%. On analysis using *t* – test there were no significance difference between plants of the same species

Conclusion: The plant extract of *C. africana* and *B. helenae* from Kagera and Njombe demonstrated dose dependent uterotonic effect. The plant extract of C. africana and B, helenae contains the following phytochemical groups, saponins, carbohydrates, cardiac glycosides, phytosterols, phenols, tannins and tarpenoids phytochemical groups in *C. africana* and *B. helenae* from Kagera and Njombe as well. The plant extracts also use the following receptors to induce uterine contractions oxytocin receptor (OTR), cholinergic receptor (ChR),

adrenergic receptor (ADR) and endotheline receptor (ETR). However, there was no statistical difference in phytochemicals and uterotonic effects between the plants of the same species from Kagera and Njombe, Tanzania.

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background

Local herbs have been used for treatment and prevention of diseases for many years worldwide. In many rural areas of Tanzania local herbs are used as traditional medicines to induce labour and shorten labour time and are range between 40 and 90%. This is due to the Social-cultural acceptance, financial constrain of most people in the rural areas and poor health services in the rural areas as the health facilities are located far from some village communities which hinder the provision of proper health services to women, children and disabilities (Mugisha and Origa, 2007) . In Tanzania the average of pregnant mother who have at least one antenatal visit is 87% and who have 4 visits is 42.8% also the average number of pregnant women who gives birth in health facilities is 50.2% (UNDP, 2013). Despite these percentages, yet the expected mother use local herbs for induction of labour and shortening labour time due to lack of knowledge and financial resources to support them during labour as many women in Tanzania live in the rural areas where the access to medical service is of great challenge (UNDP, 2013). This behavior which cause many complications to the mother and her fetus which includes but not limited to fetal distress, ruptured uterus, maternal distress, post-partum hemorrhage (PPH). These complications contribute to maternal and fetal death. These complications are associated with higher dose of the herbal extracts they are prepared without proper dosage and the type and the concentrations of the bioactive compounds found in it are not known since there is no information about them (Dika *et al.*, 2017).

The study which was conducted in Mwanza region revealed that most of the women in Mwanza use local herbs for management of labour. They use them at various stage of labour. They use local herbs during labour to manage complication such as poor uterine atone, prolonged labour. Other pregnant mother use local herbs before the onset of labour for labour induction and other use those herbs for shorten labour time and they use it after active stage of labour in order to avoid caesarian section deliveries (Dika *et al*, 2017).

The study that was conducted in Nigeria showed that the traditional healers/ traditional birth attendants use the local herbs such as *Commelina africana, Sida corymbosa and Vernonia amygdalina*. The extract of these plant when tested for induction of the uterine contraction on the uterine cells cultured on the gel it gave positive result as they have uterotonic effects similar to the positive control (Attah *et al*, 2012).

The use of medicinal plants in Africa and many developing countries has risen the interest to World Health Organization (WHO) which lead to the thorough studies on these medicinal plants (local herbs) and to increase effort of documenting the information of these plants as the traditional healers and traditional birth attendants (TBAs) does not keep records on these plants and they pass information verbally from generation to generation (Kaido *et al*, 1996). Researches have been directed towards proving the therapeutic claims explained by traditional healers on these plants. Most people who use medicinal plants for treating various diseases depend on traditional healers for preparation of remedies and for information on the recipe for preparation so if these traditional healers have to be organized they could bring advantages to the community (Kaido *et al*, 1996).

In South Africa, many black women use the local herbal remedies as antenatal medication, for induction of labour, for management of third stage of labour and for post-partum hemorrhage prevention. The most common plants used for the above functions are Isihlambezo (*Agapanthus africanus*). The pregnant women use the remedies prepared by growing this plant in water container and drink the water morning and night in order to ensure health child, to prevent the new born baby from developing bowel problem and to facilitate the easy delivery of placenta. Icimamilo (*Pentanisia prunelloides*) is another plant used by Zulu and Xhosa. Root decoction of this plant is used to facilitate the expulsion of retained placenta (Veale *et al* 1992). All these two plants showed to have the capacity to induce uterine contraction by acting particularly on the uterine smooth muscles. All these plants have induced the uterus similar to the induction by Oxytocin hence if used in higher doses during labour they can cause uterine hypertonia (Kaido et *al.*, 1996).

The study that was conducted in Uganda also shows that there are about seventy five medicinal plants and one fungus (toadstool mushrooms – Tricholomaticeae family) which are used for induction of labour. Most of these plants are used as food which includes but not limited to garlic, cassava, sorghum and tea. This study shows that cassava tea have the ability to induce uterine contractions which means that local herbs have the potential ability to induce uterine contraction and can be used for management of various complications associated with poor uterine contraction (Mugisha and Origa, 2006).

The study that was conducted in Mwanza also showed that pregnant women use local herbs to induce labour and shorten labour time. The plants used which identified by both local and botanical names are ginger (*Zingier officinale*), onions (*Allium cepa*) and neem (*Azadirachta indica*) and mgagani (*Cleome gynadra*). Other plants used were only identified by their local names such as akabindizi, ekinunulizi, enyabashumi, mshana and msuana (Dika *et al.*, 2017).

The study done in Kagera region shows that there are many plants used as herb medicines by women for abortion. These plants were tested for induction of uterine contraction and found to induce contraction (Nikolajsen et *al.*, 2011). Although these plants are used for abortion they are the same plants used for labour induction and shortening of the labour time. These plants may have different bioactivities due to differences in soil type, season when they are harvested, weather of the particular area and the post-harvest processes **(**Rasch and Kipingili, 2009**)**. Therefore, these plants need analysis of phytochemicals and to determine their ability to induce uterine contraction.

1.2. Statement of the problem

The use of local herbs to induce labour, shortening of labour time, and treating minor diseases during pregnancy and treating complication that arise during labour is of WHO interest as many communities in the world commonly use these local remedies worldwide. The local herbs used in Kagera region Tanzania, were analyzed for efficacy in inducing uterine contraction. *C. africana* and *B. helenae* used for management of labour showed they are capable of inducing both frequency and strength of the contraction (Nikolajsen et *al*, 2011). These plants are also found in other regions of Tanzania such as Njombe and they are used as

vegetables which can cause adverse to pregnant mother such as abortion and pre term deliveries. However the types and the amount of phytochemicals responsible for induction of uterine contraction can be affected by the area where these plants are growing, seasons and time of harvesting among other factors (Deng *et al* 2010). This may cause the user to consume plant materials with phytochemicals which can induce uterine contraction or plant materials without phytochemicals responsible for induction of uterine contraction depending on the geographical location. The TBAs or individual pregnant mother may use these plants found in any region in Tanzania but no study was conducted to analyze if plants from different geographical location in Tanzania have the same potency. Thus, the objective of this study is to compare phytochemical constituents and bioactivity of *C. africana*, and *B. helenae* and to determine the receptors used by the plant extract to induce uterine contractions collected from Kagera and Njombe in Tanzania.

1.3 Rationale of the study

It is anticipated that the findings of this study will help to increase ethnobotanical repository of the plants with uterine contraction activity based on their geographical locations so as to avoid adverse effects such as abortion which will associate with consumptions of the plants with uterotonic effects by pregnant woman as these plants used in Kagera region are not used in other geographical location such as Njombe for management of labour and its complications such as post –partum hemorrhage. Also the findings of this study will help to understand the receptors which are targeted by the bioactive compounds from *C. africana* and *B. helenae* which will help proper management of pregnant women if we want to combat its effect when using them while pregnant.

1.4. Research questions

1. Are the extracts of *C. Africana* and *B. helenae* from Kagera and Njombe in Tanzania capable of inducing uterine contraction?

2. Are the strength of inductions caused by *C. africana* and *B. helenae* from Kagera different from that caused by *C. africana* and *B. helenae* from Njombe?

3. Do the extracts of *C. africana* and *B. helenae* from Kagera and Njombe in Tanzania have the same phytochemical constituents?

4. What are the receptors used by the bioactive compounds from plant crude extract to induce uterine contraction?

1.5. OBJECTIVES

1.5.1. Broad objective

To compare the phytochemical constituents and bioactivity of herbal medicines used for induction of labour from Kagera and Njombe in Tanzania

1.5.2 Specific objectives

1. To investigate the phytochemicals present in *C. africana* and *B. helenae* collected from Kagera and Njombe regions

2. To determine the ability of the plant extracts of *C. africana*, and *B. helenae* from Kagera and Njombe regions to induce uterine contraction.

3. To determine if there is a difference in the induction strength between C. africana from Kagera and that from Njombe also between B. helenae from Kagera and that from Njombe

4. To determine the receptors used by the bioactive compounds found in these plants to induce uterine contraction.

CHAPTER TWO

2.0. LITERATURE REVIEW

2.2. Biology of the uterus

The uterus is the key organ of female reproductive system. It is on average approximated to be 7cm long and 4 – 6cm wide, it is pear shaped and the most muscular organ of female reproductive system. It is divided into three sections, namely the cervix, the isthmus and corpus uteri and each part has the specific function (Symonds and Symonds 1998). The uterus is made up of three layers the endometrium, myometrium and the perimetrium. The myometrium is the middle layer which is responsible for uterine contraction as it composes muscle cells myometrial smooth muscle cells (myocytes) to form smooth muscles of myometrium. The smooth muscles are arranged in undefined layers which makes the contraction forces to occur in different directions which help the uterus to flexible to assume any shape. Throughout the pregnancy it protects the fetus with the help of myometrium. During labour the uterus through myometrium contract in rhythmic fashion so as to deliver the fetus and placenta as well and also to control severe breeding after delivery (Gruber, O'Brien 2011). The collagen fibers are the major component of ECM that surrounds smooth muscle cells. These collagen fibers provide attachment site for smooth muscle cells also transmit the force generate by smooth muscle cell contraction. The smooth muscles are arranged in two layers, longitudinal and circular layer the contraction of these layers lead to dilatation and shorten of the organ respectively hence facilitate the expulsion of uterine contents during delivery (Csapo 1962) (Gruber, O'Brien ,2011).

2.3. Uterine contraction mechanism

There are many pathways through which the uterine contraction will be induced. Myosin light chain kinase (MLCK) pathway which involve the Phosphorylation and dephosphorylation of myosin for contraction and relaxation respectively by regulating calcium ion concentration. The intracellular calcium concentration is regulated through receptors such as endotheline receptors (ET1, ET2 and ET3), Calcium channels (VOC and AOC), passive entry, prostaglandin (PG) receptors of membrane (E2, F2α). The increase in intracellular Calcium cause the contraction of the myometrial smooth muscles which is caused by stretch of smooth muscle cells (Sakurada et al 1998) and Young (2007).

Acetylcholine (ACh) and Oxytocine stimulate the cell to produce second messenger D-myoinositol 1,4,5-triphosphate (IP3) under the effect of Phospholipase C which **is** coupled to the OTR by G – protein on the plasma membrane constituent phosphatidylinositol 4,5-bisphosphate (PIP2). When IP3 **is** released it induce sarcoplasmic reticulum (SR) to releases calcium ion thus increasing the concentration $Ca2^{+}$ and resulting in cell contraction (Stull et al, 1991) (Gruber, O'Brien, 2011). Diacylglycerol (DAG) produced when IP3 is released is used as the second messenger which induce the production of arachidonic acid (AA) which also induce cell stretch hence uterine contraction occurs. The uterine contraction utilizes many receptors so as to ensure that the expulsion of the uterine content is achieved within a required time and prevent other complication such as retained placenta, post-partum hemorrhage and uterine atone. The mechanism is summarized in the figure 1 bellow Lopez (2007) and Gruber, O'Brien, (2011).

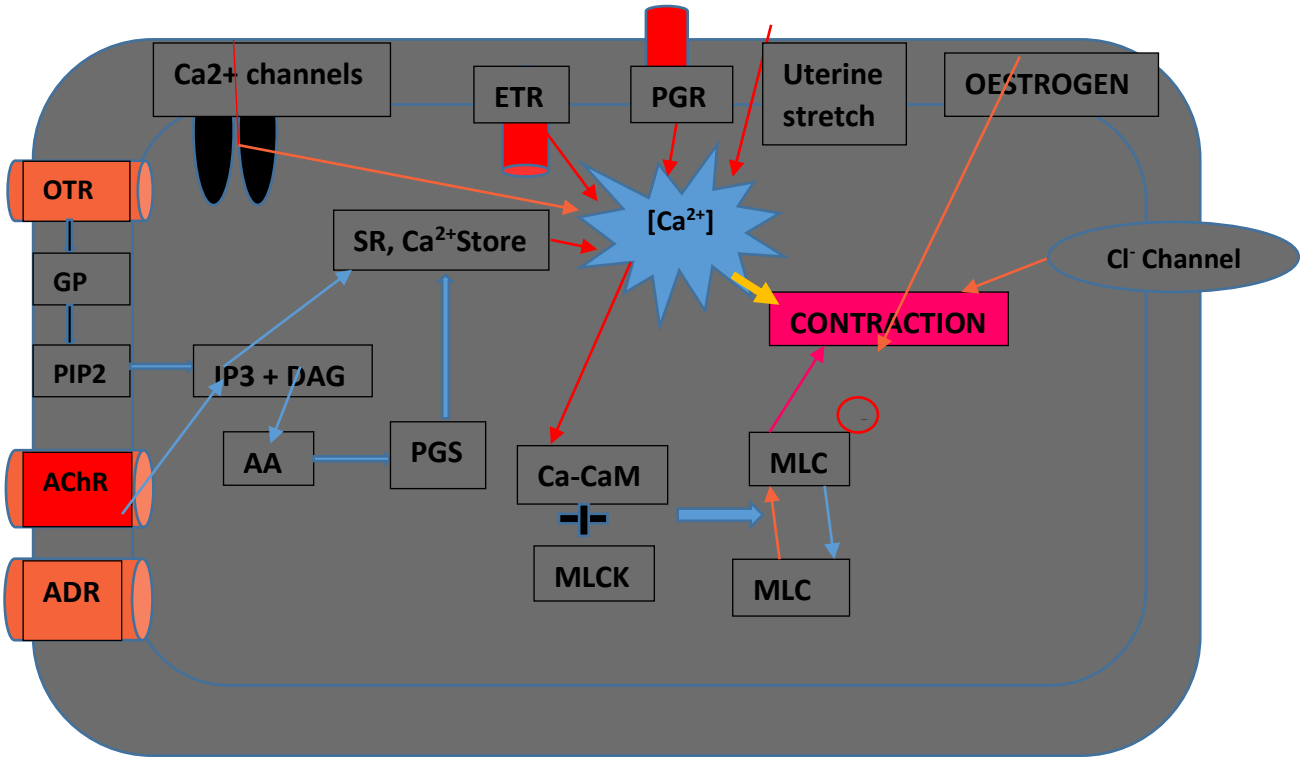


Figure 1: Shows the signal transduction of uterine contraction mechanism Gruber, O'Brien, (2011)

2.1. The use of local herbs for management of labour

The study that was conducted in Kagera region (Tanzania) reported that, the traditional birth attendants use plant extracts from many plant species for induction of labour, strengthening of the uterine contraction and to facilitate the management of of both the third stage labour and post – partum hemorrhage (Nikolajsen et *al*, 2011). The remedies are extracted from these plants species *Commelina africana, Cassia mimosoides, Desmodium barbatum, Manihot esculenta, Oldenlandia corymbosa, Obetia radula, Ocimum suave, Ricinus communis, Rubia cordifolia, Sphaerogyne latifolia, Triumfetta microphylla, Vernonia amygdalina*. The extract of these plants were tested if they can cause contraction on the rat uterus and both plants caused contraction at the concentration of 1.4mg/mL and above (Nikolajsen et *al*, 2011).

2.4. *Commelina africana*

Commelina africana (figure 2) is the medicinal plant used in most areas of Africa for management of gynecological problems. In South Africa it is used for treating infertility, dysmenorrhea and management of labour. It is the perennial plant with small canary yellow flowers that grow well in sandy and loam soil. The flowering season of this plant is between October and April (Obermeyer 1985). In Tanzania, *C. africana* is widely distributed in Kagera, and Njombe regions and less distributed in Ruvuma**,** Tanga and Coastal regions. Leaves are variable, oblong to linear, flat or folded, up to 120 mm long but usually smaller, glabrous or glabrescent to variously hairy. This is used by the TBAs for induction of labour and management of labour during delivering. The TBAs use it for prevention of Post-Partum Hemorrhage (PPH), management of poor uterine contraction and for complete delivery of the placenta (Nikolajsen et *al*, 2011).

Figure 2: ***C. africana*** **(with voucher number at ITM Herbarium Mg& SH 2 Mg& SH 4)**

2.5. Biophytum helenae

Biophytum helenae (figure 3) is the perennial and annual herbaceous plant from Oxalidaceae family. It is distributed in tropical and sub-tropical areas worldwide particularly in tropical East Africa. It has simple stem and sometimes shortly branched at the apex and shortly branched at the apex. The leaves are sensitive to touch and the leaflets closing together upwards when touched (Christine 1971). In Tanzania the plant is distributed in Kagera, Kigoma and Njombe regions particularly in shady places, waste land, river-banks, under damp thickets and at elevations up to 250 metres. *B. helenae* is used by traditional healers and TBAs for management of many conditions including treatments of venereal diseases, infertility, induction of labour, and management of post-partum hemorrhage and poor uterine contraction during labour (Nikolajsen et *al*, 2011).

In East Asia the decoction of the roots and stem of this plant is used for treatment of various diseases such as diabetes mellitus, tuberculosis, sore throat, abscesses, chronic wounds and fever. It is also used to treat inflammatory diseases, disease of pregnancy and to diminish female libido (Burrows and Willis 2005).

Figure 3*: **B. helenae*** **(with voucher number at ITM Herbarium Mg & SH 1 and Mg & SH 4)**

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study design

This was experimental study design which involved phytochemical screening and determination of uterotonic effects of C. *africana* and *B. helenae* whole plants (roots, stem and leaves) from two regions, Njombe and Kagera.

3.2. Study area and population

The samples of *C. africana* and *B. helenae* whole plants which includes roots, stem and leaves used by TBAs for preparation of remedies for induction of labour was collected from Kagera and Njombe. These two regions are among the known geographical area where these plants are found as per botanist reviews and have different climatic condition. The sample was collected in May 2020 under the assistance of a botanist. The sample was transported to MUHAS where extraction, phytochemical screening and assessment of uterotonic were conducted.

3.3. Chemicals and equipment

Chemicals: ethanol (Loba Chemie – laboratory reagents and fine chemicals, Mumbai, India), PSS, diethystilbestrol (Sigma Aldrich, Darmstadt, Germany) and phytochemical screening reagents.

Equipment: Buchi Rotavapor RE 111 with a water bath at 40^0C and 14 mbar (BÜCHI Labortechnik AG, Flawil Switzerland) vortex mixer, analytical balance, tissue bath, separating funnels, retort stand.

Animals: Laboratory rat uterus were used for determination of ex vivo uterine contraction. Three female Wistar rats which were sexually matured of six weeks and average body weight of 179.3 g ± 28.9 were used as per Sengupta (2013). The rats were obtained from ITM animal house.

3.4. Preparation of the sample

The whole plants (roots, stem and leaves) of *C. africana* and *B. helenae* were dried under shade in a well-ventilated area then using electric hammer mill dry samples were grounded into course powders and the powders were stored in jars which are air-tight and placed in a cool place waiting for extraction.

3.5. Extraction of crude extracts

The protocol developed by Tibuhwa was used for extraction of crude extract from the sample of the plant materials. The procedure used ethanol: water solution at a ratio of 8:2 which means 80% ethanol and 20% water without heating. This technique is also termed as cold maceration procedure (Tibuhwa, 2014). The grinded powders of *C. africana* (350 g), *B. helenae* (150 g) from Njombe and *C. africana* (250 g) and *B. helenae* (350 g) from Kagera were soaked separately in ethanol water solution (8:2) for 48 hours at room temperature. By using the cotton wool and Buchner funnel the mixture of powder and ethanol water solution was filtered and the residue was extracted again for 24 hours to ensure thorough extraction of crude extract. By using rotary evaporator at a temperature of 40 to 50°C and vacuum environment the crude extracts were concentrated to remove solvents. Further drying was achieved by freeze drying of the extracts to remove traces of water. The dried extracts (figure 4) were stored at -20°C until the time used for phytochemical screening and assessment of uterine contraction.

Figure 4: **Crude extracts of *C. africana* of *B. helenae* of Kagera and Njombe**

3.6. Induction of uterine contraction experiment

This procedure was performed by using tissue/organ bath technique. The organ bath contained the physiological saline solution (PSS). The PSS to be used is the De Jalon's solution the following compositions, NaCl 9g/L, KCl 0.5g/L, 0.5g/L of glucose, 10.9 HEPES, $CaCl_2$, NaHCO3 1g/L and 4M NaOH used for pH adjustment according to Nikolajsen et *al.*, 2011).

The laboratory rat was subcutaneously injected 2 mg/kg body weight of diethystilbestrol 48 hours before testing for uterine contractions. The induction was repeated 24 hours after the first induction to ensure maximum increases of estrogen level of the rat so as to increase myometrial activities of the rat uterus including facilitation of uterine contractility by preventing myometrial atrophy (Setyawati *et a*l., 2018). After 48 hours the rat was sacrificed by cervical dislocation. The euthanized rat was evaluated if it is completely non responsive to noxious stimuli by hind paw pinch before dissection and remove of uterus (Boston University Medical School 2007). The dissection area and required equipment which includes two dissecting scissors (large and small), two dissecting forceps, dissecting dish, dissection pins, and aluminum tissue clips. The rat was dissected and the uterus was isolated. This procedure

was performed under the guidance of animal science expert from pharmacology department and the uterus was placed in the petridish which contain physiological saline solution (PSS).

The isolated uterus was longitudinally cuts to expose the inner part of the uterus so as to enhance maximum exposure of the uterotonic receptors during testing for uterine contractions. Throughout the process the uterine tissue was hydrated with PSS (Arrowsmith *et al.*, 2018). The uterine strip of the induced rat was mounted on 2 g tension found in an organ/tissue bath which contain 20 mL of PSS at 37˚C, pH of 7.4 and continuously gassed with 95% oxygen and 5% carbon dioxide for recording of the tissue contraction (figure 5). The tissue was equilibrated for 30 minutes then the PSS was changed. Then tissue was left for 2 minutes in the bath to allow the spontaneous phasic contraction. After the tissue was equilibrated and the contractions was stopped then the induction of the rat uterine contraction using the crude extract of plants was performed by using different concentrations of crude extracts. Each of these concentration of the crude extract 0.04, 0.14, 1.44, 2.14 and 3.14 mg/ml was added in the tissue bath in triplicates and the percentage of uterine contraction was calculated by relating with that of oxytocine as the 100% reference percentage of each concentration. The percentage force of contraction was calculated by taking the height of the kymograph of the crude extract divide by that of oxytocine multiplied by 100%. The mean percentage contraction was calculated from each concentration of crude extract (Nikolajsen et *al*, 2011).

Figure 5: **Rat uterine mounted in the curvet of the tissue bath ready for testing of uterine contraction**

3.7. Determination of the receptors used to induce uterine contraction

The receptors used by the plant extracts to induce uterine contraction were determined by using antagonist drugs which block the drugs to binds to the uterine receptors so as to induce contraction. For oxytocine receptors, atosiban was used by adding 300 µg (Godwin *et al.*, 1996). The cholinergic receptor antagonist atropine 0.5 mg, the adrenergic receptor antagonist salbutamol 0.5 mg (Sato et *al.*, 1996) and endotheline receptor antagonist, nifedipine 1.0 mg was used according to (Dechanet *et al.*, 2011).

All receptor antagonists were added together in the tissue bath and left for 10 minutes then 2.14 mg/mL of crude extract was added and observed if the uterine contraction was occurred. If the contraction occurs means the bioactive in the crude use other receptors rather than those used to induce contraction.

Then the solution without one, two or three receptor antagonist until remain with one receptor antagonist was prepared so as to determine the types of receptor used. Then by using receptor antagonist to determine if the receptor used by bioactive compounds to induce uterine contraction.

3.8. Phytochemical analysis (screening)

The phytochemical analysis was done to determine the type of phytochemicals found in these plants. The phytochemicals groups investigated includes alkaloids, saponins, phytosteroids, phenols, tannins, flavonoids, cardiac glycosides, protein and amino acids, tarpenoids. Each phytochemical group was screened as explained below:

Alkaloids test was performed by dissolving the crude extract into hydrochloric acid and filtered. The filtrate was tested with the following reagents. Dragendroffs reagent (potassium bismuth iodide solution if it forms red precipitate indicate the presence of the alkaloids (Yadav and Agarwala, 2011).

Saponins tests. Froth test was used to determine presence of saponins. The extract was diluted into 20 ml of distilled water and shaken in the graduated test tube if it forms 1 cm layer of forms it indicate that the extract contains saponins. Foam test also was used to test saponins by diluting small quantity of plant extract in 2 ml of distilled water and observe the forms produced if it is persistently for about ten minutes (Yadav and Agarwala, 2011).

Phytosteroids test. Small quantity of crude extract was dissolved in the chloroform then followed by two tests, Slkowski's test where few drops of concentrated sulfuric acid was be added and allow the solution to stand, the formation of brown ring indicate the presence of phytosteroids. Libermann Burchard's test. The chloroform extracted solution was treated with few drops of acetic anhydride then boiled and cooled then the concentrated sulfuric acid was

be added, the formation of bluish green solution indicate the presence of phytosteroids (Yadav and Agarwala, 2011).

Phenols testing. The crude extract was treated with 3 – 4 drops of ferric chloride solution. If the solution turned blue-black color it indicates the presence of phenols (Yadav and Agarwala 2011).

Tannins screening. The 0.5g of crude extract powder was mixed with 20ml of water in the test tube and boiled then filtered, few drops of 0.1% of ferric chloride will be added, and the formation of brownish green or blue black coloration it indicates the presence of tannins. (Yadav and Agarwala, 2011).

Flavonoids screening. This was performed by using alkaline reagent test by treating the crude extract with few drops of sodium hydroxide solution. The formation of intense yellow color which change to colorless when adding dilute acid indicate the presence of flavonoids (Yadav and Agarwala, 2011).

Cardiac glycosides. This was done by using Keller Killan test by treating the crude extract with 2ml of glacial acetic acid containing one drop of ferric chloride solution then 1ml of concentrated sulfuric acid was added, appearance of browning at interface indicate the deoxysugar characteristics of cardenolides, appearance of a violate ring bellow the brown ring and the greenish ring in the acetic acid layer confirm the presence of cardiac glycoside (Yadav and Agarwala, 2011)

Tarpenoids screening. This was done by Salkowski's test. The extract was mixed with 2ml of chloroform then followed by addition of concentrated sulfuric acid 3mls to form layers, the formation of the reddish brown colour of the interface will indicate the presence of tarpenoids (Yadav and Agarwala, 2011)

3.9. Statistical analysis

The data of induction of uterine contraction was summarized in the table to shows the amplitude (effect height) in centimeters and also in the bar chart to show the pictorial presentation of that data. The analysis of the comparison of uterine contraction effects (mean percentage of uterine contraction of each plant extract) from the two plants which collected from Njombe and Kagera was analyzed by using student *t* – test through R – Software.

The data of phytochemical screening was summarized in the table so as to shows the groups of phytochemicals which are found in the plants from the two regions to compare if they contain the same phytochemicals or not

The data for receptors used by the bioactive compounds were summarized in the table and compared if the plants from two regions have the bioactive compounds which use the same receptors.

3.10. Ethical consideration

The main ethical considerations were the legal permission to conduct research, harvest plant materials and animal welfare before and during animal experimentation. The permission to conduct research was obtained from MUHAS Ethical Review Board/Committee and permission to collect plant materials was requested from forest authorities. The rat was kept in the well ventilated room at temperature of 20˚C to 27˚C with enough supplies of water and food. Also the room was kept at 12hr light and 12 hours dark to mimic the day and night. The rat was sacrificed by cervical dislocation and the dissection to remove uterus was conducted after the rat is fully unconscious. The rat carcasses were disposed by incineration.

3.11 Data Dissemination

The finding from this study will be presented at MUHAS scientific conference and other conferences (National and International). Soft copy of the dissertation will be available through the MUHAS Repository. A manuscript will also be prepared for publication in a peer reviewed journals. Also feedback will be provided to the communities where these plants were collected.

CHAPTER FOUR

4.0. RESULTS

4.1. Phytochemical analysis

The results of phytochemical analysis revealed that the crude extract of *C. africana* and *B. helenae* whole plants from Njombe and Kagera contained saponins, phytosteroids, phenols, tannins, carbohydrates, cardiac glycosides and tarpenoids as presented in table 1 below. The analysis further revealed that alkaloids and flavonoids were absent in both plants from different region and these phytochemicals reduces the uterine contraction.

Table 1: Phytochemical constitutions of *C. africana* and *B. helenae* whole plants from Kagera and Njombe regions

Site of collection (REGION)	Phytochemical group	*C. africana* (whole plant)	*B. helenae* (whole plant)
Kaagaya village (KAGERA)	Alkaloids	-	-
	Saponins	+	+
	Phytosteroids	+	+
	Phenols	+	+
	Tannins	+	+
	Flavonoids	-	-
	Carbohydrates	+	+
	Cardiac glycosides	+	+
	Tarpenoids	+	+
Nundu village (NJOMBE)	Alkaloids	-	-
	Saponins	+	+
	Phytosteroids	+	+
	Phenols	+	+
	Tannins	+	+
	Flavonoids	-	-
	Carbohydrates	+	+
	Cardiac glycosides	+	+
	Tarpenoids	+	+

Key: (+) = present; (-) = absent; whole plant = roots, leaves and stem

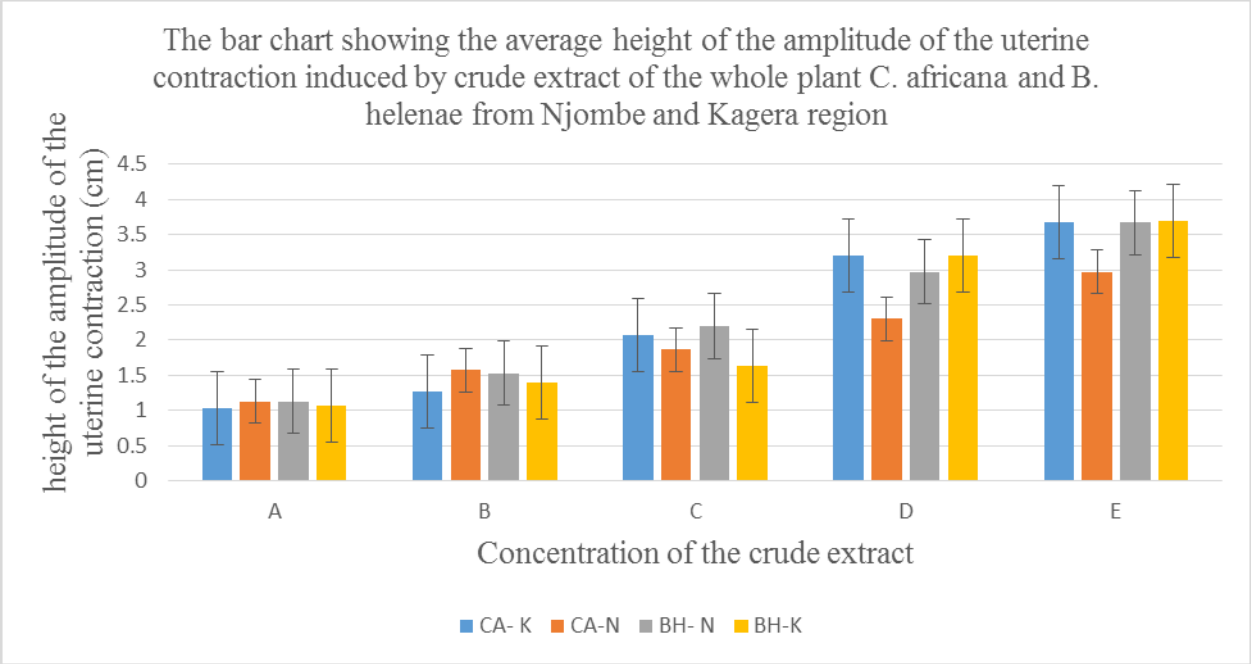
4.2. Induction of uterine contraction

The uterotonic effect of the plant extract from whole plant (stem roots and leaves) of *C. africana* and *B. helenae* was found to range from 27.2% to 97.3% and are summarized in the table 2 below and in the figure 6 below.

Table 2: Uterotonic effects of crude extract from whole plant of *C. africana* and *B. helenae*

Plant extract conc. (mg/ml)	A (0.04)	B (0.144)	C (1.03)	D (1.44)	E (2.144)
C. africana Kagera av H(cm) (%)	1.04±0.52 (27.24)	1.27±0.03 (33.16)	2.07±0.52 (54.05)	3.2±0.52 (83.55)	3.27±00.52 (95.82)
C. africana Njombe av (cm) (%)	1.13±0.31 (31.39)	1.57±0.31 (43.61)	1.87±0.31 (51.94)	2.3±0.31 (63.89)	2.97±0.31 (82.5)
B. helenae Kagera av H (cm (%)	1.07±0.52 (28.16)	1.4±0.06 (36.84)	1.63±0.52 (42.89)	3.2±0.52 (84.21)	3.7±0.52 (97.37)
B. helenae Njombe av H(cm) (%)	1.13±0.45 (29.2)	1.53±0.45 (39.53)	2.2±0.45 (56.85)	2.97±0.45 (76.74)	3.67±0.45 (94.83)

***Key**: The uterotonic effect of the crude extract of the whole plant of Commelina africana whole plant (roots, stem and leaves) from Kagera and Njombe region and Biophytum helenae whole plant from Kagera and Njombe expressed in average height (cm) ±standard deviation (SD) and percentage. Student two tailed t-test was used to identify statistical difference between plants of the same species but from different geographical locations. No statistical difference between the plants of the same species where p>0.05*



Key: CA-K= crude extract of C. africana Kagera whole plant (roots, stem and leaves), CA-N= crude extract of C. africana whole plant Njombe, BH-N= crude extract of B. helenae Njombe whole plant and BH- K= crude extract of B. helenae Kagera whole plant. A= 0.04mg/ml, B= 0.144 mg/ml, C= 1.03mg/ml. D= 1.44mg/ml and E= 2.144mg/ml.

Figure 6 : Effect of crude extract of the whole plant of *C. africana* and *B. helenae* from Kagera and Njombe

The figure 6 above is the bar chart which represents the height of the amplitude of the uterine contraction in cm after exposed to the different concentration of the crude extract of the whole plants of *C . Africana* and *B. helenae.* The contractions were concentration dependent as the concentration increase also the uterine contraction increases. The contractions were maximum at the concentration of 2.144 mg/ml which almost equivalent to that caused by carbachol

Table 3: Statistical analysis of uterotonic effect between plants of the same species (C. africana from Njombe with that from Kagera and B helenae from Njombe with that from Kagera

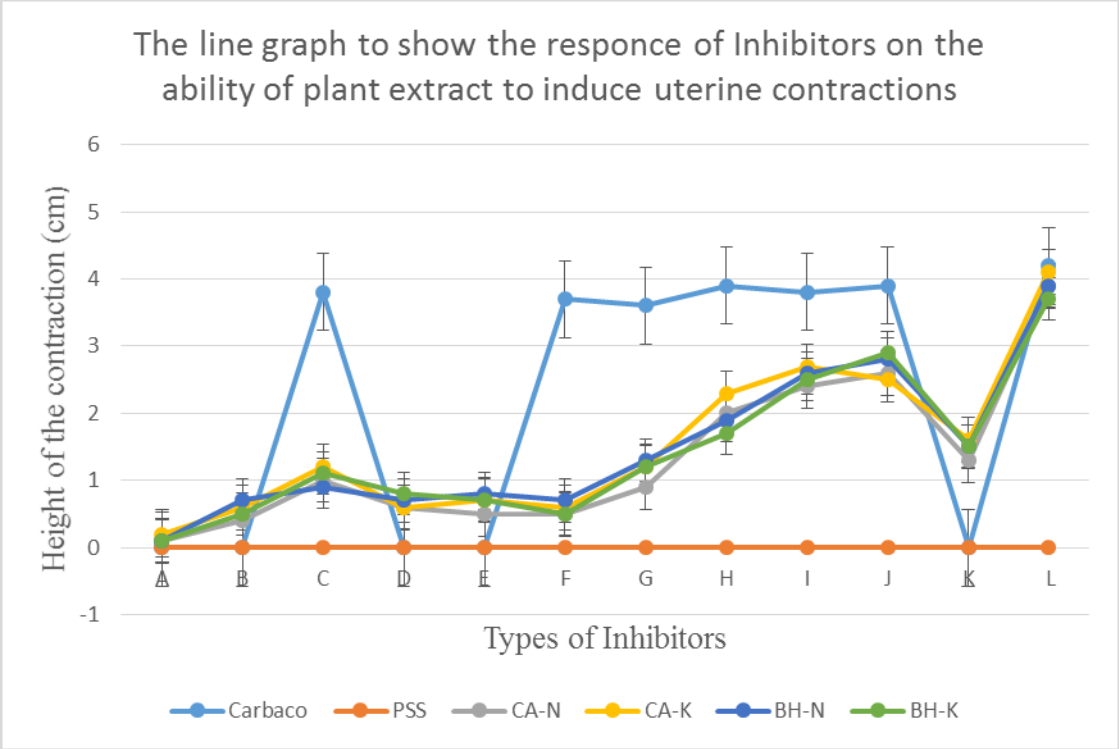
	C. africana	*B. helenae*
t	0.46485	-0.14293
Df	6.599	6.599
P	0.657	0.8899
Mean difference	0.28	0.1

The table 3 above shows the statistical analysis of the uterotonic effects between the plants of the same species using R software where the effects shows no significance difference as the p value for C. africana was 0.657 which is greater than 0.05 and that of B helenae was 0.8899 which also greater than 0.05 thus no statistical significance. The mean difference for uterine contractions of C. africana from Kagera and that from Njombe was 0.28 cm and the mean difference of for uterine contractions of B. helenae from Kagera and that from Njombe was -0.1cm

Key: p = p value, df = degree of freedom and t = two tailed test statistic

4.3. Determination of the receptors used to induce uterine contraction

Figure 7: Bar chart of the height of uterine contraction vs inhibitors at different combination



Key: *Carbacol is the positive control, PSS (physiological saline solution is the positive control, CA-N is the crude extract of C. africana from Njombe, CA-K is C. africana from Kagera, BH-N is B. helenae from Njombe. A to L represents the types of Inhibitors used where A (OTR, ChR, ADR and ETR), B (OTR, ChR and ADR), C (ChR ,ADR and ETR), D (OTR, ADR and ETR), E (OTR and ChR), F (ChR and ADR), G(ADR and ETR), H (ChR), I (ADR), J (ETR), K (OTR), L (No Inhibitor)*

Figure 7 **:** Line graph that shows the height of uterine contraction vs inhibitors at different inhibitor combination

From figure 7 above, the line graph shows the uterotonic effect of the crude extract of the whole plant of C. africana and B. helenae from Kagera and Njombe when influenced by different inhibitors. The contraction was affected by inhibitors as the contraction tend to decrease when inhibitors was applied. Oxytocine receptor (OTR) antagonist, cholinergic receptor antagonist (ChR), adrenergic receptor (ADR) antagonist and endotheline receptor

(ETR) antagonist was used. The contractions were reduced when the crude extract was applied after the antagonist applied. And both the antagonist mention above reduce the uterotonic effect of the plant extract. This suggests that the plant extract mediate uterine contraction through OTR, ChR, ADR and ETR. Also there were weak contraction of approximately 0.02% of effect when compared with carbacol (positive control) observed when all four inhibitors was used it also suggest that other receptors such as calcium channel was also mediate the uterotonic effect of the plant extract.

CHAPTER FIVE

5.0. DISCUSSION

5.1. Induction of uterine contraction

The results presented above (Table 2) show that the plant extracts have the ability to induce the uterine contraction. At the highest concentration, the extracts induced maximum response on uterine contraction similar to that induced by carbachol (1µg/ml) used as the positive control drug. The height of maximum peak caused by induction with carbacol was considered as 100% effect and was used to calculate the uterotonic effect of the plant extracts by comparing their maximum peaks. The finding of this study also showed that the uterotonic effect is dose - dependent as it increases with the increase in the concentration of the crude extract which used to induce the contraction of the rat uterus. Similar findings were also obtained on the study aimed to screen plants which are used for inducing abortion in Kagera Tanzania. Many plants including *B. helenae* and *C. africana* were identified to have uterotonic activities and can induce abortion (Nikolajsen *et al*, 2011). The study on *Calotropis procera* shows that the extract from this plant at a concentration of 1.30 mg/ml have the capacity to induce uterine contraction with the amplitude of 21.9±4.2 cm compared to that caused by oxytocine which gives the maximum amplitude of 22.7±2.3 cm with no significance difference (Shamaki *et al.*2014). The results obtained in this study also showed that there was no significant difference in the uterotonic effects between the crude extracts of the same species and extracts from different plant species from Kagera and that from Njombe. This signifies that the two plant species studied have the same potential of inducing uterine contraction regardless of their geographical location. These findings are supported by the results of phytochemical screening (table 1) which showed that both plant species from Njombe and Kagera have the same phytochemical groups.

This confirms that the geographical location did not affect the presence of bioactive compounds associated with induction of uterine contraction. Similar findings were also reported in the study conducted in Uganda on *Bidens pilosa* that compared the uterotonic effect of the extract from *B. pilosa* from the different geographical distribution in Uganda with

no significant difference on the uterotonic effect of *B. pilosa (p>0.05)* (Kamatesi *et al*, 2007).

5.2. Phytochemical screening

The findings of this study reports the presence of saponins, carbohydrates, cardiac glycosides, phytosterols, phenols, tannins and tarpenoids phytochemical groups in *C. africana* and *B. helenae* from different geographical locations. Results further revealed absence of alkaloids and flavonoids in these two plants (Table 1). Previous studies showed that presence of alkaloids and flavonoids were associated with inhibition of uterine contraction whereas presence of other phytochemical groups was associated with induction of uterine contraction. Both plant species did not contain alkaloids and flavonoids. The absence of these two groups of phytochemicals support the uterotonic effect of the plant extract as those two groups were proven with other studies conducted on *Colotropis procera* that they reduce the uterine contraction on the uterine tissue (Shamaki *et al* 2015).

The study conducted on *Withania somnifera* by Visweswari *et al*, (2013) and that conducted on *Paris polyphyla Sm. var yunnensis* by Guo *et al*, (2008) reported that saponins were responsible for uterine contraction. Hence the uterine contraction induced by the crude extract of the whole plants of *C. africana* and *B. helenae* collected from Kagera and Njombe may be contributed by the presence of saponins in these two plant species.

The findings also show that the crude extract of the whole plants of *C. africana* and *B. helenae* from both two regions contains cardiac glycosides and tarpenoids. These two groups of phytochemicals also have the potential effect on uterine contraction as they increase its effect. The study conducted on *Calotropis procera* by El-Shemy (2017) shows that cardiac glycosides and tarpenoids have ability to induce uterine contraction. This is also similar to the study that conducted by Moustafa *et al*, (2010) on cardiac glycosides and tarpenoids. Hence the presence of the cardiac glycosides and tarpenoids in the crude extract of the whole plant of *C. africana* and *B. helenae* from Kagera and Njombe support the uterotonic activities of these plants.

5.3. Receptor determinations.

The findings of this study showed that the uterotonic activities were reduced when four antagonists were used (Figure 7). The oxytocine receptor- antagonist (atosiban), adrenergic receptor antagonist (salbutamol), cholinergic receptor antagonist (atropine) and endotheline receptor antagonist (nifedipine) were used. These four antagonists block the effects of uterine contraction induced by the crude extracts of the whole plant of *C. africana* and *B. helenae.* The contraction reduced from above 82.5% to less than 4.7% uterotonic effect. This suggests that the uterotonic effect of the two plants species was mediated by oxytocine receptor (OTR), cholinergic receptor (ChR), endotheline receptor (ETR) and adrenergic receptor (AdR). The presence of saponins, tarpenoids and cardiac glycoside as the phytochemical groups in the crude extract of the two plant species from Kagera and Njombe suggest that the extract mediate uterine contraction through different uterine receptors. This is due to the fact that saponins, tarpenoids and cardiac glycosides differ in chemical compounds also they induce the uterine contractions by using different receptors. The study like this was done on *Ananas comosus* to determine the receptors and the findings showed that the extract of *A. comosus* edible parts induce uterine contraction through serotonin receptor by using Ketanserin (serotonin receptor inhibitor) (Monji *et al*, 2016). The crude extract was capable to induce the uterine contraction even when four inhibitors was used this means that there were also other receptors which mediate the uterine contraction. The extract contain cardiac glycoside which mediate uterine contraction through $3Na^{+}/Ca^{2+}$ channel/ pump. Hence the presence of many receptors to mediate uterine contraction of the induced by crude extract of the whole plant is due to the presence of many phytochemical groups.

5.3. Limitations of this study and mitigations

I. Lack of sophisticated equipments such as uterine contraction transducer makes this study to be difficult and subjected to errors.

II. Mitigations. I used smoked drum to record the effect of the contraction

CHAPTER SIX

6.0. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

In this study the plant extracts from *C. africana* and *B. helenae* showed the uterotonic activities in rat uterine. The effects were dose dependent as the uterine contraction increased with the increase in the concentration of the crude extract from the whole plant of *C. africana* and *B. helenae* from two regions. However, there was no significant difference on the induction of the uterine contraction between the extracts from two plant species collected from different geographical locations. This study also suggest that the receptors that used by plant extracts to induce uterine contraction were OTR, AChR, ADR and ETR. The screening of plant extracts revealed the phytochemicals contained to include: saponins, tarpenoids and cardiac glycosides but absence of alkaloids and flavonoids. The present phytochemicals may play major role in the induction of uterine contraction.

6.2. Recommendations

The findings of this study give rise to the following concerns:

I. More studies to determine the phytochemical concentrations and types of each phytochemical is required.
II. Health education to the user of these plants is required as these plants has the activities similar to Carbacol/ Oxytocine so the TBAs and community at large need to be aware of the timing to augment the labour.
III. More studies on the receptors other than those studied, as there were uterine contractions even in the presence of all antagonists.
IV. More studies on the bioactive ingredients responsible for uterine contractions is required
V. More studies for compounding of the bioactive ingredients and proper dosing is required.

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