

**UNIVERSITY OF KWAZULU-NATAL**

**Contractile effects of *Gunnera perpensa* and  
*Rhoicissus tridentata* bioactive extracts in isolated  
rat uterine muscles**

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**Contractile effects of *Gunnera perpensa* and *Rhoicissus tridentata* bioactive extracts in isolated rat uterine muscles**

**By**

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**Submitted in fulfilment of the requirements for the degree of Master of Medical Sciences in Human Physiology in the Discipline of Human Physiology, School of Laboratory Medicine and Medical Sciences, College of Health Sciences**

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School of Laboratory Medicines and Medical  
Sciences, College of Health Sciences**



## DECLARATION

I, Sinenkosi Carol Dube, student registration number **212560095** hereby declare that the thesis entitled:

**“Contractile effects of *Gunnera perpensa* and *Rhoicissus tridentata* bioactive extracts in isolated rat uterine muscles”**

is the result of my own investigation and research. This work has not been submitted in part or in full for any other degree or to any other university. All information from research articles is properly referenced in the text.

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2/03/2015  
**Date:**

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To my nieces and nephews, know that the roots of education are bitter, but the fruits are sweet.

**To my family**

## **PLAGIARISM DECLARATION**

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## LIST OF ABBREVIATIONS

<i>A. africanus</i>	<i>Agapanthus africanus</i>
Ach	Acetylcholine
ACTH	Adenocorticotrophin hormone
ATR	Atropine
ANOVA	One-way analysis of variance
ATP	Adenosine triphosphate
<i>C. thalictroides</i>	<i>Caulophyllum thalictroides</i>
Ca <sup>2+</sup>	Calcium ion
cAMP	Cyclic adenosine monophosphate
<i>C. miniata</i>	<i>Clivia miniata</i>
Cl <sup>-</sup>	Chloride
CRH	Corticotrophin releasing hormone
DCM	Dichloromethane
<i>G. perpensa</i>	<i>Gunnera perpensa</i>
<i>H. ceres</i>	<i>Helichrysum ceres</i>
HPA	Hypothalamic-pituitary-adrenal axis
IL-8	Interleukin 8
K <sup>+</sup>	Potassium
KCl	Potassium chloride



<i>L. chuanxiong</i>	<i>Ligusticum chuanxiong</i>
MeOH	Methanol
MLCK	Myosin light chain kinase
<i>M. ciliatum</i>	<i>Monechan ciliatum</i>
Na <sup>+</sup>	Sodium
NaCl	Sodium chloride
NO	Nitric oxide
<i>P. americana</i>	<i>Persea americana</i>
<i>P. prunelloides</i>	<i>Pentanasia prunelloides</i>
PGE <sub>2</sub>	Prostaglandin E <sub>2</sub>
PGF <sub>2α</sub>	Prostaglandins F <sub>2α</sub>
PPH	Postpartum haemorrhage
PR	Progesterone receptors
PTHrP	Parathyroid hormone related peptide
ROC	Receptor operated channels
<i>R. tridentata</i>	<i>Rhoicissus tridentata</i>
TGF-β1	Transforming growth factor-β1
<i>T. kirilowi</i>	<i>Tricosanthes kirilowi</i>
<i>T. capensis</i>	<i>Typha capensis</i>
<i>S. birrea</i>	<i>Sclerocarya birrea</i>
VGC	Voltage gated channels

## ABSTRACT

### Introduction

*Gunnera perpensa* and *Rhoicissus tridentata* are commonly used by women to induce labour, expel the retained placenta and prevent postpartum haemorrhage. Reports indicate that *G. perpensa* has lactogenic activity which is mediated through binding to acetylcholine receptors. However, the current study investigated the contractile effects of *G. perpensa* and *R. tridentata* extracts in isolated rat uterine muscles in an effort to validate the indigenous knowledge systems since traditional birth attendants claim that these plants are effective in alleviating gynaecological complaints and not toxic.

### Materials and methods

*G. perpensa* roots were purchased from vendors at Durban market and *R. tridentata* species were collected from Silverglen nursery. The plant species were identified by the botanist, Miss Christina Potgieter at the Bews herbarium in University of KwaZulu Natal, Pietermaritzburg campus where the voucher specimen was deposited. The extraction was performed in Prof Van Heerden's laboratory at the Chemistry department, Pietermaritzburg, University of KwaZulu Natal. The current study investigated the contractile effects of *G. perpensa* and *R. tridentata* extracts using the isolated rat uterine muscles. Stilboestrol-treated rats were sacrificed by decapitation and 2-3 cm of the uterine muscles were isolated and suspended in an organ bath containing aerated de Jalon's buffer maintained at 32 °C. Each muscle strip was subjected to an applied resting tension of 1.0 g and allowed to equilibrate for 45-60 min. Rhythmic contractions in tissues incubated in the absence of plant extracts or standard drugs served as a control. During treatment with various doses of plant extracts, force and rate of uterine muscle contractions were recorded using powerlab system. The effects of the plant extracts were compared to with the standard drugs, oxytocin and acetylcholine. Data obtained from the plant extract-treated uterine muscle strips, in addition to those obtained from the non-treated control and combination treatment were calculated as a percentage with reference to the standard drug treatment. All data was analyzed using Graphpad instat software version 5 and is presented as means  $\pm$ SEM.

## Results

Extraction with different solvents afforded *G. perperisa* and *R. tridentata* root and leaf methanol crude extracts. Further fractionation of *R. tridentata* root methanol crude extracts yielded dichloromethane (DCM), methanol and acetone fractions.  $\beta$ -sitosterol was isolated from DCM fraction while compound 285, asiatic-arjunolic acid, resveratrol glycoside, morin rhamnoside, quercitrin and catechin were identified from the acetone fraction.

Treatment with *G. perperisa* and *R. tridentata* root and leaf methanol crude extract evoked significant ( $p < 0.05$ ) increases in the rate and force of uterine muscle contractions. These results were comparable to the effects induced by standard drugs oxytocin and acetylcholine. Co-administration of *R. tridentata* root methanol crude extract and oxytocin induced significantly ( $p < 0.05$ ) greater effects when compared to the treatment with *R. tridentata* root methanol crude extract alone. Furthermore, methanol and acetone root fractions exhibited significant increases in the rate and force of contractions. However, the acetone extract was more potent. Treatment of uterine muscles with  $\beta$ -sitosterol, compound 285 and asiatic-arjunolic acid amplified the contractility of this tissue. Resveratrol glucoside as well as morin rhamnoside exhibited significant decreases in uterine muscles whereas other compounds such as quercitrin and catechin completely abolished the contractions.

## Discussion

The current study has shown that *G. perperisa* and *R. tridentata* root and leaf crude and bioactive extracts possess uterotonic effects although the mechanisms cannot be explained from the current observations. Literature evidence, however, indicates that some plants enhance parturition by acting on calcium stores, thus releasing this ion which is involved in the initiation of myometrial contractility. Therefore, we speculate that these effects may be mediated in part via calcium mobilization. On the other hand, the relaxant effects exhibited by resveratrol glucoside and morin rhamnoside may be mediated via activating the voltage-gated  $K^+$  channels or increasing levels of cAMP. The inhibitory effects exhibited by catechin and quercitrin may be in part attributed to the toxicity of these drugs. Therefore, the findings of this study suggest that *G. perperisa* and *R. tridentata* crude extracts induce hyperstimulation of the uterus. Hence, these

extracts may play significant roles in parturition. Furthermore, *R. tridentata* contains both uterotonic and tocolytic constituents which contribute to the effectiveness of this plant in managing many gynaecological complications.

## **Conclusions**

Our results suggest that *G. perpersa* and *R. tridentata* root and leaf extract can be used to induce labour and to manage other gynaecological complications.

## **Recommendations**

Further studies are required to elucidate the mechanisms of action of *G. perpersa* and *R. tridentata* extracts.

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## CHAPTER 1

### INTRODUCTION/ LITERATURE REVIEW

#### 1.0 Background

Throughout the years, nature's diversity has been reliable in the management of gynaecological disorders worldwide. Despite the increasing practice of traditional therapies, documentation on indigenous healing systems is insufficient. Previously the healing systems were passed down verbally from generation to generation, therefore the information may be lost with the succeeding generations. Researchers have, however, developed an interest in characterizing the plants, isolating the active compounds and preserving the information through publishing articles. Literature evidence indicates that some of the frequently-used Southern African medicinal plants have beneficial therapeutic effects suggesting that new drugs which may be easily accessible to the general population may be produced from plants. The World Health Organization (WHO) recommends the use of uterotonics to initiate and accelerate the third stage of labour. Decoctions of *Gunnera perpensa* and *Rhoicissus tridentata* have been extensively used by women to promote childbirth. Therefore, to add on the currently available information this study was undertaken to investigate the contractile effects of *G. perpensa* and *R. tridentata* bioactive extracts on isolated rat uterine muscles.

## 1.1 Introduction/ Literature review

Medicinal plants have long been used by populations in low income countries to facilitate childbirth (Attah *et al.*, 2012). Reports indicate that about 70-80% of the women use folk medicine during pregnancy to promote uterine muscle contractility and prevent postpartum haemorrhage (Steenkamp, 2003; Bafor *et al.*, 2010). Scientific reports indicate that most of the pregnancy-related complications mainly result from poor uterine muscle contractility implying that uterine contractions play a vital role in childbirth (Katsoulis *et al.*, 2000). Current methods used to enhance uterine muscle contractility and prevent postpartum bleeding include administration of uterotonic agents such as oxytocin, prostaglandins (PGF<sub>2α</sub>, PGE<sub>2</sub>) and ergometrine (Selo-Ojeme, 2002; Rath, 2009). However, oxytocin remains the drug of choice due to the efficacy in promoting childbirth and prevention of postpartum haemorrhage (Stanton *et al.*, 2013). Literature evidence indicates that co-administration with other drugs increases the efficacy of synthetic oxytocin (Alfirevic *et al.*, 2007). Conventional drugs are effective at low concentrations, but are however toxic at higher doses and extra care may be required (Alfirevic *et al.*, 2007; Bafor *et al.*, 2010). Therefore, there is a greater need for new therapeutic approaches which provide alternative and cost-effective treatment (Gurib-Fakim, 2006). Central Africa has a diverse range of plants containing bioactive compounds that possess many therapeutic effects (Okigbo *et al.*, 2009). Hence, folk medicine has been widely used to treat many ailments including gynaecological and pregnancy-induced complications. The different concoctions prepared from medicinal plants provide supplements that enhance foetus growth and are also beneficial in promoting uterine contractility (Sewram *et al.*, 1998). Reports indicate that plant extracts of the *G. perperna*, *R. tridentata*, *Clivia miniata*, *Agapanthus africanus*, *Typha capensis* and *Pentania prunelloids* are prescribed by traditional healers to pregnant women to hasten childbirth and expel placenta (Sewram *et al.*, 1998; Veale *et al.*, 2000; Khan *et al.*, 2004; Bafor *et al.*, 2010; Gruber and O'Brien, 2011). These reports indicate an increase in scientific evaluation of plants with oxytocic properties. Accordingly, the current investigations were designed to investigate the contractile effects of *G. perperna* and *R. tridentata* bioactive plant extracts on isolated uterine muscles.

## 1.2 The uterus physiology

The human uterus is a hollow-shaped muscular tissue consisting of myometrium, a bundle of smooth muscle fibres and the endometrium lining which plays a major role during follicular phase of the menstrual cycles. The endometrium degenerates and develops under hormonal regulation (Maruyama *et al.*, 2013). Oestradiol which is a form of oestrogen is synthesized at high concentrations during pregnancy for maintenance of the placenta and foetus (Grosset and Grosset, 2004). Another hormone involved is progesterone, a cholesterol-derived hormone produced by the corpus luteum and trophoblasts during the first trimester and placental cells when corpus luteum has degraded (Bradshaw and Bradshaw, 2011). Progesterone prepares the endometrium for the fertilized egg implantation by maintaining the uterine lining. However, if the egg is not fertilized, the levels of progesterone decrease followed by menstrual cycle which lasts for about 3-5 days (Henriet *et al.*, 2012). During pregnancy, the cervix is closed and uterus consisting of the maternal and foetal tissues is quiescent. However, when the foetus has fully developed, the cervix dilates and the uterus contracts vigorously allowing the expulsion of a baby (Duggan *et al.*, 2007). The uterine contractility is influenced by the electrical stimulation of the myometrial cells (Aguilar and Mitchell, 2010). The contraction-relaxation mechanisms of the uterine, like those of a smooth muscle are regulated by changes in ion concentrations which are controlled by the secondary messengers. Influx and efflux of ions such as calcium ( $\text{Ca}^{2+}$ ), potassium ( $\text{K}^+$ ), sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) change the polarity of the plasma membrane. Increased levels of intracellular  $\text{Ca}^{2+}$  triggers uterine contractions through calmodulin-dependent myosin light chain kinase (MLCK) activity and is modulated by G-protein coupled receptors (Webb, 2003). Studies have shown that some plant-derived compounds exert uterotonic effects through increased extracellular  $\text{Ca}^{2+}$  influx and release of intracellular  $\text{Ca}^{2+}$  from stores (Guo *et al.*, 2008). Hence, research on the mechanisms through which plants cause or enhance myometrial contractility is essential.  $\text{Ca}^{2+}$  mobilization and stimulation of efflux from myometrial cells is inhibited during pregnancy, consequently, the uterus becomes inactive. However, during parturition a process through which the foetus is expelled, calcium is mobilized and as a result the uterus shifts from inactive to an active state by developing coordinated contractility. The process of parturition consists of four phases which involve transformation of the uterine from inactive to active state. These four phases are discussed below.

### 1.3 Human pregnancy

The processes of pregnancy begin with fertilization of an ovum by spermatozoa forming a zygote, a fertilized egg. Under normal conditions, the zygote is propelled by cilia from the fallopian tube to the uterus where implantation to the endometrium occurs (Maruyama *et al.*, 2013). The foetus is nourished in the uterus until birth and is involved in the birth processes through the activation of hypothalamus-pituitary-adrenal (HPA) axis (Snegovskikh *et al.*, 2006). Foetal hypothalamus secretes corticotrophin releasing hormone (CRH) which triggers the release of adenocorticotrophin hormone (ACTH) by the pituitary gland to the adrenal glands. Consequently, cortisol and oestrogen precursors are released into circulation by the adrenal glands (Schindler, 2005). Steroidal hormones lead to increased production of oxytocin, prostaglandins and placental CRH, thus initiating labour. The human parturition is regulated by prostaglandins and oxytocin and their respective receptors (Golightly *et al.*, 2011). The foetal membranes (amnion and chorion) and decidua ensure uterine maturation and also synthesize prostaglandins which play a major role in the expulsion of the foetus. Contractions of the myometrial cells are greatly influenced by the oxytocin, a hormone which is produced in the hypothalamus. Oxytocin enhances labour by increasing the production of prostaglandins and the number of gap junctions (Gimpl and Fahrenholz, 2001; Snegovskikh *et al.*, 2006). Studies suggest that *Paris polyphylla* contain compounds such as steroidal saponins which possess oxytocin-like effects. The mechanism through which this compound exerts the effects is, mediated, in part via  $\text{Ca}^{2+}$  influx from extracellular fluid (Guo *et al.*, 2008). Some plant constituents act by enhancing the effect of the oxytocin and prostaglandins through increased receptor expression or direct binding to uterotonic receptors (Salleh and Ahmad, 2013). However, further studies are still required to investigate the efficacy of plant-derived compounds in enhancing childbirth. The herbal remedies used during pregnancy are prescribed by traditional healers during the last trimester to prepare the uterus for parturition, a process consisting of four phases which are quiescence, activation, stimulation and involution phase.

### 1.3.1 Quiescence phase

The uterus is maintained in a quiescent state by progesterone and a variety of inhibitors throughout pregnancy (Gökdeniz *et al.*, 2013). Progesterone is produced by the corpus luteum during the first trimester of pregnancy and is used to treat preterm labour (Meis *et al.*, 2003). However, due to luteal regression, the placental cells continue to synthesize progesterone. Dimeric progestins from rhizomes of *Ligusticum chuanxiong* have been shown to exert progesterone-like effects which are mediated through activation of the progesterone receptors (Lim *et al.*, 2006). Hence, *L. chuanxiong* may have the potential to enhance the effects of progesterone in case of preterm labour. Progesterone controls the myometrium by interacting with specific myometrial progesterone receptors. Progesterone interaction with transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) results in an increase the rate of parathyroid hormone related peptide (PTHrP) transcription. PTHrP is a protein which binds specifically to PTHrP receptors in the myometrium activating the G proteins, thus increasing the intracellular levels of cAMP which inhibits  $\text{Ca}^{2+}$  influx and mobilization from the stores resulting in uterine quiescence. Reports indicate that nitric oxide (NO) inhibits the myometrial contractility in rats and humans. Therefore, NO may provide the therapeutic effects for conditions such as preterm labour and dysmenorrhoea. However, increased concentrations of NO attenuates myometrial sensitivity to oxytocin (Okawa *et al.*, 2004; Mohamed *et al.*, 2009). Other inhibitors involved in the quiescence phase include relaxin, calcitonin gene-related peptide, prostacyclin  $\text{I}_2$ , vasoactive intestinal polypeptide and endogenous  $\beta$ -adrenergic agonists (Challis *et al.*, 2000). The effects of the aforementioned inhibitors are mediated via increasing the levels of intracellular cAMP or through lowering the availability of calcium channels (Gökdeniz *et al.*, 2013). Some plants contain uterotonic and tocolytic compounds hence, the desired effects depend on the preparation of the herbal remedy. The uterine relaxant effects of some folk medicine play a major role during pregnancy by shifting the uterus towards quiescence, thus averting preterm labour (Williams *et al.*, 2013). Researchers have shown interest in isolating the active ingredients in plants to further elucidate their mechanisms of action. We speculated that the outcome of the current study will show the effects of the active ingredients contained by our plant of interest. However, at term, the myometrial quiescence phase switches to activation phase.

### 1.3.2 Activation phase

During activation phase, the synthesis of oestradiol and cortisol increases thus resulting in the expression of contraction-associated proteins, oxytocin and prostaglandins receptors. The number of gap junctions amplify allowing cell-to-cell coupling (Snegovskikh *et al.*, 2006). The myometrial contractions are mediated through ATP-dependent binding of the myosin to actin filaments. Intracellular  $\text{Ca}^{2+}$  increases and initiates the contractions. G-proteins couple cell membrane receptors to effector enzymes and ionic channels such that activation of prostaglandins  $\text{E}_2$  receptors promotes uterine muscle relaxation through  $\text{G}_{\alpha s}$ , adenylyl cyclase or cAMP signal transduction pathways. In contrast, oxytocin receptors couple to  $\text{G}_{\alpha q}$ ,  $\text{G}_{\alpha i}$  or phospholipase C pathways leading to the elevation of inositol-1,4,5-trisphosphate which promotes the release of  $\text{Ca}^{2+}$  from the sarcoplasmic reticulum, causing myometrial contractions during the stimulation phase of parturition (Lye, 1996; Shmygol *et al.*, 2006). However, some herbal remedies have been shown to have beneficial effects in the stimulation of uterine contractions (Meier and Wright, 2000). *Chromoleana morii*, *Linum usitatissimum* and aquatic sedge have been shown to contain prostaglandin-like compounds which stimulate the myometrial contractions (Groenewald and Westhuizen, 1997). Reports suggest that medicinal plants such as *A. africanus* and *C. miniata* activate the uterus through different mechanisms (Tripathi *et al.*, 2013). *A. africanus* stimulates myometrial contractility by binding to muscarinic receptors and also by promoting prostaglandin synthesis. Similarly, *C. miniata* stimulates myometrial contraction through the activation of cholinergic receptors and prostaglandin synthesis (Gruber and O'Brien, 2011; Tripathi *et al.*, 2013). Another mechanism by which some of the medicinal plants induce exert their contractile effects is through mimicking the action of oxytocin. The activation phase prepares the myometrium to respond optimally to the stimulants that provoke myometrial contractility during labour.

### 1.3.3 Stimulation phase

The CRH together with the agonists, oxytocin and prostaglandin  $E_2$  and  $F_{2\alpha}$  stimulate myometrial contractility (Kota *et al.*, 2013). Circulating oestradiol triggers the release of oxytocin from the posterior pituitary into circulation. In response to that, oxytocin and CRH promote the secretion of prostaglandins. When prostaglandins bind to their respective receptors labour is initiated. The biochemical events that occur during labour are similar to that of inflammatory response. Labour is initiated when cytokines and migration of neutrophils into the uterus occur. When the cytokines such as interleukin (IL-8) attract the neutrophils into the foetal membrane and cervix, uterus remodeling and maturation result (Snegovskikh *et al.*, 2006; Terzidou, 2007). Some studies speculated that the uterotonic effects induced by plants may be mediated in part via prostaglandin synthesis, thus mobilizing  $Ca^{2+}$  from calcium stores or by causing cervical ripening (Gruber and O'Brien, 2011). Hence, there is a necessity to investigate the mechanisms in which *G. perpersa* and *R. tridentata* promote the uterine muscle contractility. However, the fourth phase of parturition is the involution phase which occurs after the baby and the placenta have been delivered.

### 1.3.4 Involution phase

Following events primarily influenced by oxytocin, the uterus returns to normal state (Terzidou, 2007). Involution phase involves the loss of collagen and the reduction of the size of the uterus. The processes of tissue loss and repair may take about 30 days to restore the normal functioning of the uterus. Reports indicate that the number of lactations affect the cervical and uterine involution (Sheldon, 2004). However, many complications may arise during gestation period or at term and most of these complications are related to poor uterine muscle contractility. In this study, we investigated the beneficial effects of *G. perpersa* and *R. tridentata*, plants that are commonly used by women to avert pregnancy-related complications. To try and understand whether our plants have beneficial effects in pregnancy, we investigated the contractile effects of the active compounds found in *R. tridentata* on isolated rat uterine muscles.



## **1.4 Pregnancy-associated complications**

During pregnancy the cardiovascular, renal, respiratory and haematological systems are involved. In response to the demands of pregnancy, maternal adaptations including vascular alterations, protection of uterus from starvation, removal of waste products and preparation for labour occur (Norwitz *et al.*, 2005). However, these adaptations may cause complications such as oedema, hypertension and dyspnea. Other pregnancy complications are those associated with uterine contractility. Herbal remedies are increasingly used to alleviate many ailments including gynaecological complaints (Malan and Neuba, 2011; Tripathi *et al.*, 2013). These herbal remedies exert their effects through different mechanisms, as reports indicate that some plants extracts have oxytocin-like effects while others act by increasing the production of cyclooxygenase metabolites. However, the paucity of information on the mechanisms of action of these plants and their ability to cause maternal and or foetal toxicity is a major health concern (Veale *et al.*, 2000). Hence, more research is needed to investigate the toxicity of these plants. This study partly investigated the toxicity of our plant extracts on isolated uterine muscles.

### **1.4.1 Preterm labour**

Approximately 3 million neonatal deaths are reported annually due to delivery of premature babies (Beck *et al.*, 2010). Preterm birth refers to birth before 37 weeks' gestation. Preterm births occur spontaneously however, gestational age and environmental factors have an influence in the progression of this disorder (Romero *et al.*, 2002). The causes of preterm birth may result from maternal or foetal complications. These complications which commonly occur between 22-32 weeks are caused by inflammation related to infection. Antepartal bleeding, stress as well as uterine over distention, a condition caused by development of multiple foetuses in the uterine is associated with the preterm births (Challis *et al.*, 2000). Traditional healers prescribe some medicinal plant extracts to combat pregnancy complications such as preterm births. Reports indicate that plants such as *Ficus exasperata* have the potential to alleviate such complications (Bakor *et al.*, 2010). Therefore, further research to evaluate the properties of the bioactive compounds extracted from medicinal plants that are used by traditional healers is essential (Gruber and O'Brien, 2011).

### 1.4.2 Abnormal labour

Abnormal labour is defined as a difficult labour or slow progression of labour. This condition may be caused either by abnormalities in uterine muscle contractility or size differences of the pelvis and the foetus (Vayssière *et al.*, 2013). Currently, oxytocin remains the drug of choice in averting abnormal labour by increasing the frequency and intensity of the uterine muscle contractions, thus preventing prolonged labour (Duncan and McEwan, 2004). In areas where there is continued use of medicinal plants as a source of treatment, water crude extracts of plants such as *Luffa cylindrica* are still being used (Hostettmann *et al.*, 2000). These extracts cause strong uterine contractions immediately after oral administration. The extracts of *Monechan ciliatum* and *Tricosanthes kirilowi* are also used in the case of abnormal labour. *M. ciliatum* extracts have been shown to mimic oxytocin while *T. kirilowi* extracts strengthen the uterine contraction and enhance the response of the myometrium to oxytocin (Gruber and O'Brien, 2011). Another gynaecological disorder that is one of the major causes of maternal mortality is postpartum haemorrhage.

### 1.4.3 Postpartum haemorrhage

Postpartum haemorrhage (PPH), the most common cause of maternal mortality is excessive loss of blood after giving birth (Selo-Ojeme, 2002; Carroli *et al.*, 2008). PPH is currently treated using uterotonic agents which include oxytocin, ergotalkaloids, and prostaglandins (Miller *et al.*, 2004; Kaingu *et al.*, 2011). However, tea made from the leaves of the raspberry bush (*Rubus ideas*) has been used for centuries as a stimulant that primes the uterus for efficient contraction during labour (Ticconi and Lye, 2002). Many other plant extracts such as silk tree bark (*Albixia julibrissin*), safflower (*Carthamus tinctorius*), motherwort (*Leonurus sibiricus*), and yarrow (*Achillea millefolium*) have been reported to have oxytocic properties and are effective in managing such disorder (Aoki *et al.*, 2005). Literature indicates that herbal remedies for gynaecological complications such as infertility and dysmenorrhea have long been prescribed by traditional healers to women.

#### 1.4.4 Dysmenorrhoea

Other gynaecological complications such as infertility and dysmenorrhea, a condition which is associated with an increased uterine tone negatively impacts the quality of life of many women in sub Saharan Africa. Women with primary dysmenorrhea have been shown to have high levels of prostaglandins  $F_{2\alpha}$  ( $PGF_{2\alpha}$ ) which in turn increases the uterine muscle contractility (Chiwororo and Ojewole, 2009). Roots and rhizomes of *Caulophyllum thalictroides* (blue cohosh) have been used to relieve menstrual cramps and ease pains associated with delivery of a baby. Decoctions of blue cohosh are used by women during the third trimester to prepare the woman for childbirth. However, reports indicate that *C. thalictroides* contains vasoactive glycosides and an alkaloid which is known to cause toxic effects on the myocardium (Dugoua *et al.*, 2008).

#### 1.4.5 Hypertension

Hypertension is a common complication of pregnancy resulting from the accumulation of fluid in the body due to sodium and water retention. Indeed, the maternal weight gain increases during pregnancy due to oedema caused by imbalance between oncotic and hydrostatic pressure in the capillaries. Hypertension is a major risk factor for some pregnancy-related complications such as preeclampsia, cardiovascular, renal diseases and placental abruption (Seely and Maxwell, 2007; WHO, 2010). The commonly used treatment for hypertension in pregnancy includes methyldopa and labetalol. The blood pressure thresholds of pregnant women for beginning with antihypertensive treatment are higher than of non-pregnant (Norwitz *et al.*, 2005). Herbalists manage hypertension and many pregnancy-related complications with decoctions made from medicinal plants. This is due to high cost and unavailability of conventional treatment to the populations in developing countries (Frishman *et al.*, 2004). Studies in our laboratory have shown that plant extracts of *Persea americana* Mill (Lauraceae), *Helichrysum ceras* S. Moore (Asteraceae), *Ekebergia capensis* Sparrm (Maliaceae) and *Sclerocarya birrea* (A. Rich) Hochst (Anacardiaceae) possess antihypertensive properties (Ojewole *et al.*, 2007; Musabayane *et al.*, 2008; Gondwe *et al.*, 2008; Kamadyaapa *et al.*, 2009). However, their effects on pregnancy-induced hypertension need to be probed.

#### 1.4.6 Placental abruption

Placental abruption is most likely to occur at 20 weeks of gestation. In this case the placenta separates from the uterine wall, leading to malnutrition of the foetus. Among other complications, hypertension has been shown to be a major risk factor for placental abruption (Norwitz *et al.*, 2005). Hypertension involves systemic vasospasm leading to poor perfusion and thus ischaemia, a condition that affects the placental blood flow, maternal cardiovascular system and renal function. Therefore, management of hypertension during pregnancy is essential. The primary goal of management of hypertension is to prevent the pregnancy-related cardiovascular complications and death (Phyllis, 2003). Conventional methods have been widely used to manage hypertension through anti-hypertensive drugs or non-pharmacological approach. These include angiotensin II inhibitors, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, calcium channel blockers, alpha-adrenergic blockers and more. However, many plants have been used in folk medicine to maintain blood pressure near normal, thus restoring normal body function (Tabassum and Ahmad, 2011). In our laboratory, we have shown that some medicinal plants such as *Syzygium aromaticum*, *S. birrea*, *P. americana* and *H. ceres* possess antihypertensive properties (Ojewole *et al.*, 2007; Musabayane *et al.*, 2008; Gondwe *et al.*, 2008; Kamadyaapa *et al.*, 2009; Ojewole *et al.*, 2010).

#### 1.5 Treatment

The use of medication by pregnant woman is highly monitored worldwide. Drugs such as oxytocin, PGF<sub>2α</sub>, PGE<sub>2</sub> and ergometrine are widely used to promote uterine contractility and to prevent postpartum bleeding (Vandongen and Groot, 1995). Dinoprostone and misoprostol are prostaglandin analogues are used to stimulate cervical dilation and uterine muscle contractions (Kreiser *et al.*, 2004; Hofmeyr and Gülmezoglu, 2008). However, there are limitations with these drugs, for example, administration of prostaglandin analogues prior to cesarean section or myomectomy, increases risks for uterine rupture (Plested and Bernal, 2001; Frishman *et al.*, 2004). On the other hand, traditional birth attendants administer medicinal plants decoctions to women for pregnancy-related conditions and these plants are effective (Kaingu *et al.*, 2011). Some standard drugs are derived from plants, showing that plants play an important role in the

health care system. Ergometrine is derived from the naturally occurring substance ergot, the alkaloid-containing product of the fungus, *Claviceps purpurea*, which grows on grain. Studies have shown that medicinal plants contain bioactive compounds which possess many therapeutic effects and are beneficial in childbirth (Tripathi *et al.*, 2013). Bioactive constituents of medicinal plants such as alkaloids, tannins, flavonoids and phenolics compounds isolated from *Asparagus racemosus*, *Clusia abyssinica*, *Clerodendrum myricoides*, *Ehretia cymosa*, *Leucas calostachys*, *Toddalia asiatica*, *Rubia cordifolia*, *Spermacoce princeae*, *Carrisa edulis* and *Ajuga remota* are beneficial in pregnancy (Jeruto *et al.*, 2008; Pascaline *et al.*, 2011). However, to add on the currently available information, this study investigated the effects of *G. perpersa* and *R. tridentata* bioactive extracts on uterine muscles.

## 1.6 Medicinal plants

Herbal medicines have been used over millennia in the treatment of various diseases. The use of herbal remedies by pregnant women has been a common practice in developing countries. Many plants have been found to possess oxytocic properties and are used to nourish the foetus, induce labour, prevent postpartum bleeding and treat other gynaecological complications. Presently, interest in the use of herbal remedies is growing because they are considered to be cheap and believed to possess few side effects. The plants used in the current study were selected based on their frequently use by pregnant women.

### 1.6.1 *A. africanus* (L) Hoffing (Liliaceae)

*A. africanus*, commonly known as an African lily, belongs to the *Liliaceae* family (Figure 1). The roots of this plant are used to prepare decoctions that are prescribed by traditional healers to pregnant women. This concoction is usually taken orally or rectally from six months of pregnancy to ensure a healthy childbirth and easy expulsion of the placenta (Veale *et al.*, 1992). Reports have shown that *A. africanus* induces smooth muscle contractions both in isolated ileum and uterus preparations (Veale *et al.*, 1992). Pharmacological screening of this plant showed that an aqueous extract of *A. africanus* leaves stimulate smooth muscle contractility and promote the response of the uterus to oxytocin and acetylcholine (Kaido *et al.*, 1997). Some plants have

therapeutic effects at lower concentrations, however, at higher doses act as an abortifacient. Other plants such as *P. prunelloides*, *T. capensis* and *C. miniata* have also been used to nourish the foetus and to prevent complications by aiding in the expulsion of retained placenta in man and animals. Indigenous systems of medicine have always been used even in developed countries where modern health care is practiced. However, less information is available on the pharmacological effects of some of the plants. Therefore, the current study investigated the contractile effects of *G. perpensa* and *R. tridentata* on isolated rat uterine muscles.



**Figure 1:** This figure shows the evergreen narrow leaves and purple flowers of *A. africanus* (L) Hoffing (*Liliaceae*), commonly known as African lily (Adapted from Ole *et al.*, 2012).

### 1.6.2 *Gunnera perpensa*. L. (Gunneraceae)

*G. perpensa* ugobho in Zulu is a perennial, robust medicinal plant which belongs to the *Gunneraceae* family (Figure 2). The roots of *G. perpensa* have been traditionally used to induce labour, expel the placenta after birth, milk letdown and to relieve menstrual pains (Nkomo *et al.*, 2010; Simelane *et al.*, 2010). Scientific reports indicate that *G. perpensa* contain several

bioactive compounds which makes this plant beneficial in the management of many disorders. *G. perpensa*-derived compounds such as *Z-venusol*, 1,1'-biphenyl-4,4'-diacetic acid, *Z*-lespedezic acid methyl ester, ellagic acid lactone, 3,3',4'-trimethyl ellagic acid acetone and *Z*-methyl lespedezate have been shown to possess many therapeutic effects (Brookes and Dutton, 2007; Nkomo *et al.*, 2010; Simelane *et al.*, 2012). Khan *et al* reported that *Z-venusol* isolated from *G. perpensa* induced the contractile effects on rat uterine muscle strips, however, these effects were observed after the physiological buffer had been removed from the organ bath (Khan *et al.*, 2004). Reports indicate that traditional healers use the decoctions of the root or rhizome for various ailments including rheumatic pains, stomach ailments and female infertility (Hutchings *et al.*, 1996). Steenkamp *et al* reported that *G. perpensa* has antioxidant properties which could be one of the mechanisms in which this plant use to avert disorders (Steenkamp *et al.*, 2004).



**Figure 2:** This picture shows the perennial, robust rhizome and leaves of *G. perpensa*. *L.* (Gunneraceae) (Adapted from Guiry *et al.*, 2007).

### 1.6.3 *R. tridentata* subsp *cuneifolia* (Vitaceae)

*R. tridentata*, a member of the *Vitaceae* family is widely used by traditional herbalist to treat many disorders that related to pregnancy (Figure 3) (Veale *et al.*, 1992). The herbal remedies prepared from this plant are taken orally by pregnant women to prevent premature delivery and growth of abnormal babies (Morris and Mdlalose, 1991). Root extracts of *R. tridentata* have a potential to stimulate and increase uterine contractility. *P. prunelloides* and *A. africanus* (uhlakahla) are plants which are commonly used by Zulu people to relieve inflammation, viral and bacterial infections and also to hasten labour and to treat gynaecological complications (Yff *et al.*, 2002). Scientific reports have indicated that *R. tridentata*, *C. miniata* and *A. africanus* (uhlakahla) promote childbirth by triggering hyperstimulation of the uterus. However, this effect may be related with toxic effects such as foetal uterine rupture, hypoxia or tachycardia (Brookes and Katsoulis, 2006). *R. tridentata* contains several compounds with beneficial effects which have been shown to be non-toxic at the doses used by traditional healers. These findings were investigated *in vitro* using human fibroblast and monkey cell lines. However, the concentration of the uterotonic agents in this plant depends on the seasonal variation (Brookes and Katsoulis, 2006). Many bioactive compounds identified in *R. tridentata* include epigallocatechin, gallocatechin hydrate, mollisacacidin, epicatechin, oleanolic acid, prostacyandin B3 and B4, sitosterolin, as well as garlic acid. This plant is commonly used in the preparation of *isihlambezo*, a herbal remedy used by pregnancy women to prepare the uterus for childbirth. These bioactive compounds are beneficial in promoting foetal growth and childbirth. The proanthocyanidins found in *R. tridentata* are uterotonic agents and their concentration vary according to the geographical source of the plants (Brookes and Katsoulis, 2006). Various herbal remedies are prepared by traditional practitioners to avert pregnancy-related disorders, one of them being *isihlambezo*.





**Figure 3:** This picture shows *R. tridentata subsp cuneifolia* (Vitaceae) trifoliolate, leathery green leaves and grape fruits (Adapted from Hyde *et al.*, 2014).

#### 1.6.4 *Isihlambezo* preparation

*Isihlambezo*, a herbal remedy consisting of a number of uterotonic plants such as *G. perpensa*, *C. miniata*, *A. Africanus*, *Asclepias fruticosa*, *Callilepis lauroleola*, *Combretum erythrophyllum*, *Crinum* sp, *P. prunelloides*, *R. tridentata*, *Scadoxus puniceus*, *T. capensis* and *Vernonia corymbosa* has been prescribed by traditional healers to women in KwaZulu-Natal to promote uncomplicated labour and the ingredients of this concoction vary with the traditional healers (Varga and Veale, 1997). The majority of the uterotonic herbal remedies are prepared by boiling the plant material in water and taken orally during the last trimester to nourish the foetus. The concentration is increased gradually to prepare the uterus for the expulsion of the baby. However, few scientific studies have investigated and documented the efficacy, mechanism(s) of action, active principles with therapeutic effects, toxicities or safer dosages of these herbs. Accordingly, studies are required to further determine the optimum doses (Flandermeyer *et al.*, 2010). Furthermore, there is limited evidence on the safety and efficacy of these uterotonic plants. Hostettmann *et al* indicated that if the traditional knowledge is not researched and documented, the information will be lost with the succeeding generations. Therefore, the

indigenous knowledge systems still need to be investigated and recorded (Hostettmann *et al.*, 2000). Table 1 below summarizes medicinal plant extracts that have been used by populations to combat gynaecological disorders.

**Table 1:** Summary of medicinal plants used to manage gynaecological complications

Biological species	Family	Bioactive compounds	Use	References
<i>G. perpersa</i> (uGhobo in Zulu)	Gunneraceae	<i>Z-venusol</i> 1,1'-biphenyl-4,4'-diacetic acid <i>Z</i> -lespedezic acid methyl ester Ellagic acid lactone 3,3',4'-trimethyl ellagic acid acetone <i>Z</i> -methyl lespedezate	↑uterine contractility placenta expulsion wound healing milk ejection abortifaciant	(Joseph and Mafatle, 1994; Khan <i>et al.</i> , 2004; Brookes and Dutton, 2007; Nkomo <i>et al.</i> , 2010; Simelane <i>et al.</i> , 2012)
<i>R. tridentata</i> (Bushman's grape)	Vitaceae	Epigallocatechin Galocatechin Hydrate Mollisacacidin Epicatechin Oleanolic acid Prostacyandin B3 ProstacyandinB4 Sitosterolin Garlic acid	induces labour ↑uterine contractility	(Katsoulis <i>et al.</i> , 2000) (Brookes and Katsoulis, 2006; McGaw and Eloff, 2008)
<i>Ficus exasperata</i>	Moraceae	Saponins Tannins	abortifaciant ↓pre-term labour	(Bafor <i>et al.</i> , 2010; Bafor <i>et al.</i> , 2013)

(Sandpaper tree)		Flavonoids Glycosides Steroids	hypertension rheumatism arthritis bleeding wound healing	(Shagal <i>et al.</i> , 2011) (Akah <i>et al.</i> , 1998) (Ayinde <i>et al.</i> , 2007)
<i>C. miniata</i> (Umayime in Zulu)	Amaryllidaceae	Alkaloids	↑uterine contractility	(Kaido <i>et al.</i> , 1997) (Haning <i>et al.</i> , 2011)
<i>Luffa cylindrical</i> (Loofah Sponge Gourd)	Cucurbitaceae	Saponins Oleanolic acid Flavonoids Phenolics Lavonoids Ascorbic acid A-tocopherol Carotenoids Chlorophylls Triterpenoids Ribosome-inactivating proteins	hasten labour ↑placenta expulsion ↓postpartum bleeding	(Kamatenesi-Mugisha <i>et al.</i> , 2007) (Azeez <i>et al.</i> , 2013)
<i>Musanga cecropioides</i> (Umbrella tree)	Moraceae	Hydrocyanide (HCN) Total oxalate Soluble oxalate Tannins Saponin Alkaloids Phytate	↑uterine contractility hypotensive vasorelaxant	(Kamanyi <i>et al.</i> , 1991; Kamanyi <i>et al.</i> , 1992; Ayinde <i>et al.</i> , 2006) (Uwah <i>et al.</i> , 2013)

<i>Parqueti nanigrescens</i>	Periplocaceae	Flavonoids Tannin Phylobatanin Saponnin Anthraquinone Alkaloids	stimulant of myometrial contractions	(Datté <i>et al.</i> , 1996) (Adebisi <i>et al.</i> , 2004)
<i>E. capensis</i> (Cape ash)	Meliaceae	Glycosides Polyphenols Tannins Triterpenes Saponnins	Uterotonic activity	(Sewram <i>et al.</i> , 2000) (Kamadyaapa <i>et al.</i> , 2009)
<i>A. africanus</i> (African lily)	Amaryllidaceae	Flavonoids Tannins	augment labour ↑smooth muscle contractility	(Kaïdo <i>et al.</i> , 1997; Veale <i>et al.</i> , 1999)
<i>P. pruneloids</i> (Icimamlilo in Zulu)	Rubiaceae	Flavonoids Tannins	↑uterine contractility Gonorrhea Tuberculosis	(Kaïdo <i>et al.</i> , 1997)
<i>Maytenus senegalensis</i> (Isihlangu in Ndebele)	Celastraceae		treats dysmenorrhea	(Sanongo, 2011)
<i>Moringa Oleifera</i> (Clarifier tree)	Moringaceae	Glucosinolates Isothiocyanates	↑uterine contractility	(Singh <i>et al.</i> , 2008)
<i>Angelica sinensis</i> (dong quai (China))	Apiaceae		Anticancer ↓premenstrual syndrome	(Zhu <i>et al.</i> , 2012)
<i>Cimicifuga racemosa</i> (black snakeroot)	Ranunculaceae		↓post-menopausal symptoms	(Juliá Mollá <i>et al.</i> , 2009)

<i>Vitexagnus-castus</i> (Chasteberry)	Lamiaceae	Flavonoids Tannins Iridoids Diterpenoids	↓premenstrual syndrome ↓premenstrual dysphoric disorder ↓hyperprolactinaemia	(van Die <i>et al.</i> , 2013) (Ignjatovića <i>et al.</i> , 2012)
<i>Ginkgo biloba</i> (Maidenhair tree)	Ginkgoaceae		Foetal growth retardation antioxidant	(Droy-Lefaix and Packer, 1999; Rudge <i>et al.</i> , 2007; Pinto <i>et al.</i> , 2007)
<i>Annona reticulata</i> (Custard apple)	Annonaceae	Analgesic Anti-inflammatory	abortifacient	(Zaman and Pathak, 2013) (Damasceno <i>et al.</i> , 2002)
<i>Lannea edulis</i> (Mutsambatsi in Shona)		Antimicrobial activity Flavonoids Tannins	gonorrhoea diarrhoea	(Chigora <i>et al.</i> , 2007; Maroyi, 2013)
<i>Rhus longipes</i> (Inhlokotshiyane in Ndebele))	Anacardiaceae	None found	↓infertility dilate birth canal	(Gelfand <i>et al.</i> , 1985; Maroyi, 2013)
<i>A. africanus</i> (Climbing asparagus)	Asparagaceae	Steroidal saponnins Analgesic Anti-inflammatory	facilitate childbirth anti-fertility activity anti-implantation activity	(Msonthi and Magombo, 1983) (Tafesse <i>et al.</i> , 2006; Maroyi, 2013)
<i>Gymnosporia buxifolia</i> (Spikethorn)	Celastraceae	d-mannitol dulcitol	treats dysmenorrhea antioxidant activity	(Bosch, 2004)

## **1.7 Aims**

The aims of the study were to investigate the uterotonic effects of *G. perpersa* and *R. tridentata* bioactive extracts in isolated rat uterine muscles. *G. perpersa* and *R. tridentata* were selected based on the literature evidence indicating that these plants are used by women during pregnancy to hasten childbirth and to alleviate pregnancy-related disorders.

## CHAPTER 2

### MATERIALS AND METHODS

#### 2.0 Drugs and chemicals

Drugs were sourced as indicated:

diethylstilboestrol, dimethyl sulphoxide (DMSO), oxytocin, acetylcholine chloride and atropine sulphate (Sigma-Aldrich, St. Louis, Missouri, United States of America);

sodium chloride (NaCl), potassium chloride (KCl), magnesium sulphate (MgSO<sub>4</sub>), calcium chloride (CaCl), sodium bicarbonate (NaHCO<sub>4</sub>), glucose (Merck, Johannesburg, South Africa).

All chemicals were of the analytical grade.

#### 2.1 Plant material

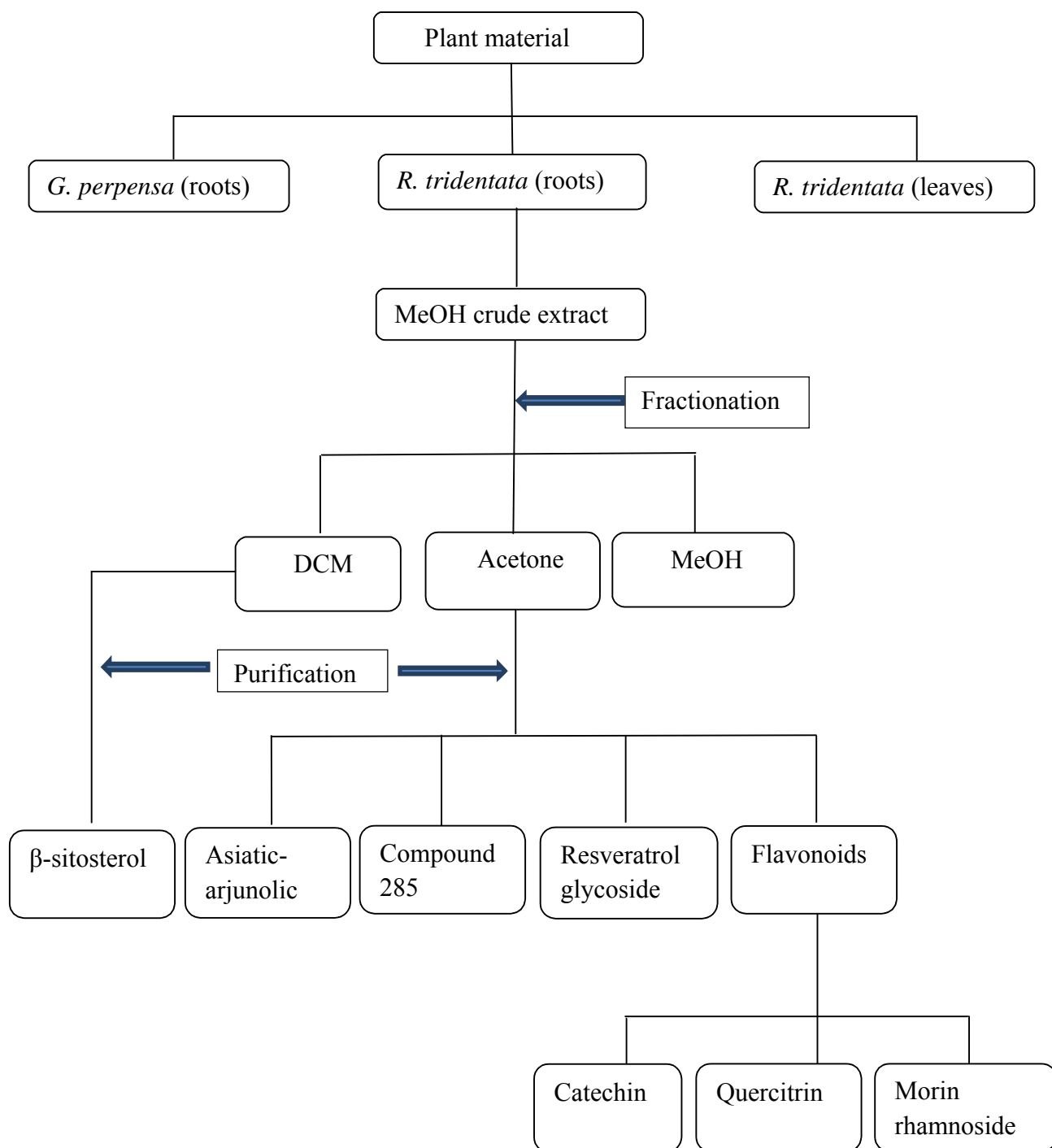
*R. tridentata* subsp. *cuneifolia* (leaves, branches and root) were collected from Silverglen nursery, Durban (KwaZulu Natal) in December 2012 and roots of *G. perpensa* roots were purchased from vendors at Durban market in January 2013. The plant species were identified by the botanist, Miss Christina Potgieter at the Bews herbarium in Pietermaritzburg Botanical Gardens where the voucher specimen was deposited. The isolation of active ingredients in these plants was performed in Prof Van Heerden's laboratory (Chemistry Department, UKZN, Pietermaritzburg campus) as shown in Figure 4.

#### 2.2 Plant extraction

The roots of *G. perpensa* were washed and sliced into small strips which were dried in the oven at 30 °C for 72 hours. Dried roots were then crushed into powder and soaked into dichloromethane (DCM), methanol (MeOH) and water. After evaporation of the solvents, 0.51 g DCM, 12.2 g MeOH extracts were obtained. Dried leaves (143.4 g) and roots (2.5 kg) of *R.*

*tridentata* were ground into powder and extracted with DCM and MeOH, for 48 hours. Resulting extracts were filtered and concentrated to dryness yielding 7.89 g MeOH and 13 g DCM extracts. The resulting *R. tridentata* methanolic crude extract was fractionated into DCM, methanol and acetone extracts. Purification of the DCM fraction yielded  $\beta$ -sitosterol while that of acetone fraction produced six bioactive active compounds which includes compound 285, asiatic-arjunolic acid, resveratrol glycoside, morin rhamnoside, quercitrin and catechin (Figure 4).





**Figure 4:** Summary showing how the plant extracts used in this study were obtained from *G. perpersa* and *R. tridentata* MeOH roots and leaves. *R. tridentata* MeOH roots were extracted with different solvents, DCM, acetone and MeOH for 48 h. DCM and acetone fractions were further purified, yielding 7 pure compounds;  $\beta$ -sitosterol, asiatic-arjunolic acid, compound 285, resveratrol glycoside and flavonoids; catechin, quercitrin and morin rhamnoside.

## **2.3 Animal studies**

### **2.3.1 Animals**

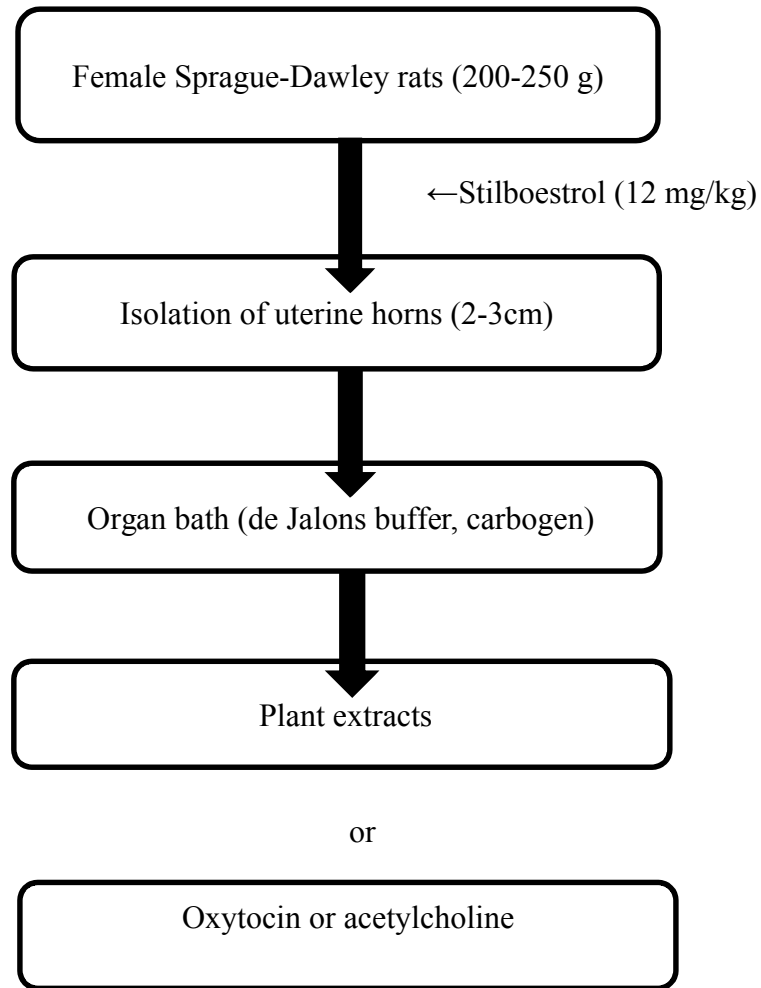
Non-pregnant female Sprague Dawley rats (200-250g) were bred in Biomedical Research Unit of the University of KwaZulu-Natal, Westville campus. The animals were kept under the conditions of constant temperature ( $22\pm^{\circ}\text{C}$ ), carbon dioxide content of  $<5000$  p.p.m., relative humidity of  $55 \pm 5\%$  and in 12-hour light and dark cycles. The animals had free access to water and standard rat chow (Meadow Feeds, Pietermaritzburg, South Africa).

### **2.3.2 Ethical considerations**

Ethical clearance was obtained from the Animal Ethics Committee of the University of KwaZulu-Natal (references: 087/13/Animal and 032/14/Animal (see Appendices A and B)).

## **2.4 Experimental Design**

The contractile effects of *G. perpensa* and *R. tridentata* extracts were investigated in quiescent rat uterine muscles isolated from stilboestrol-treated rats (Figure 5).



**Figure 5:** Experimental design used to investigate the contractile effects of plant extracts on uterine muscles *in vitro*. All data were recorded using LabChart of the Powerlab system and n=6.

### 2.4.1 *In vitro* studies

The oestrous state was induced in rats through a single intraperitoneal injection of stilboestrol (12 mg/kg, i.p.) 24 h prior to the experiments to ensure regular uterine muscle contractility. The animals were sacrificed by decapitation and uterine muscles of approximately 2-3 cm long were removed and maintained under physiological conditions as described in detail earlier by Simelane *et al.* (Simelane *et al.*, 2010). The uterine muscle strips were mounted in a 25 mL organ bath (Figure 6) chamber (AD Instruments, Bella Vista, Australia) containing freshly prepared De Jalons solution (mmol/L: NaCl, 154; KCl, 5.6, CaCl<sub>2</sub>·2H<sub>2</sub>O, 0.4; NaHCO<sub>3</sub>, 5.8 and glucose; 2.8). The De Jalons solution was continuously aerated with 5% carbon dioxide in oxygen). Each uterine muscle strip was subjected to an applied resting tension of 1.0 g, and allowed to equilibrate for 45-60 min. The tissues were incubated in new De Jalon's physiological solution every 10 minutes and allowed to stabilize. Graded concentrations is the term used to describe increasing doses of the drug in the organ bath. Graded concentration of the crude extracts (0.24-62.08 mg/mL), bioactive plant extracts (0.02-57.10 µmol/mL) and reference drugs oxytocin (0.01-109.00 nmol/mL) and acetylcholine (0.04-4070 µmol/mL) were administered after the contractions have stabilized, following which the rate and force of uterine muscle contractility were recorded. In an effort to determine whether the combination of *R tridentata* root extract and oxytocin induced an additive effect or not, the uterine muscle trips were incubated with oxytocin (1.11 nmol/mL) before being treated with various doses concentrations of the plant extracts. Contractions generated in a drug-free buffer served as the control (100%). The isotonic contractions of the uterine muscles were recorded electronically by using the Labchart of the Powerlab system (AD Instruments, Bella Vista, Australia).

### 2.5 Data analysis

Data obtained from the plant extract- and standard drug-treated uterine muscle strips, in addition to those obtained from combination treatment were calculated as a percentage with reference to the non-treated controls. The values were expressed as mean ± SEM. Statistical analysis of differences between the means of the control and treated groups were performed on the Graph Pad InStat Software (version 5.00, Graph Pad Software, Inc., San Diego, California, USA), using

one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests. A value of  $p \leq 0.05$  was considered significant.



**Figure 6:** This picture shows an organ bath which is a double walled glass chamber that maintains the tissues throughout the experimental period. Addition of drugs into an organ bath causes the isolated tissues to respond by either contracting or relaxing. These changes are detected by the transducer and recorded by LabChart of the Powerlab system.

## CHAPTER 3

### RESULTS

#### 3.0 General

This chapter gives the plant extraction data and describes the uterotonic effects of root and leaf bioactive extracts of *G. perpersa* and *R. tridentata* in isolated spontaneously contracting uterine muscle strips mounted in an organ bath containing the De Jalon's physiological buffer. Throughout the experiments, the rate and force of contractions were recorded. The tissues were exposed to various concentrations of bioactive plant extracts and/or standard drugs for 10 minutes following which the tissues were washed with De Jalon's physiological buffer and allowed to stabilize. Rhythmic contractions in tissues incubated in the absence of extracts or standard drugs served as a control. The effects of the plant extracts were compared to those obtained when the uterine muscles were treated with standard drugs, oxytocin and acetylcholine.

#### 3.1 Plant extraction

Table 2 gives the compounds that were obtained following extraction of roots and leaves of *G. perpersa* and *R. tridentata* with DCM, methanol and acetone. The bioactive compounds isolated from *R. tridentata* roots include  $\beta$ -sitosterol, compound 285, asiatic-arjunolic acid, resveratrol glycoside as well as flavonoids; morin rhamnoside, quercitrin and catechin (Table 2).

**Table 2:** Summary of the plant crude extracts and isolated bioactive compounds obtained following extraction of leaves and roots of *G. perpersa* and *R. tridentata*

Plant material	Crude extracts	Fractions	Bioactive compounds
<i>G. perpersa</i> roots	MeOH Roots (12.2 g)	-	-
<i>R. tridentata</i> leaves (143.4 g)	MeOH Leaves (7.89 g)	-	-
<i>R. tridentata</i> roots (2.5 kg)	DCM Roots (13 g)	DCM (0.9 g)	$\beta$ -sitosterol (293 mg)
	MeOH Roots (72 g)	Methanol (61.24 g)	-
		Acetone (9.96 g)	285 (2.2 mg) Asiatic-arjunolic acid (26.7 mg) Resveratrol glycoside (61 mg) Morin rhamnoside (13.6 mg) Quercitrin (21.8 mg) Catechin (2 mg)

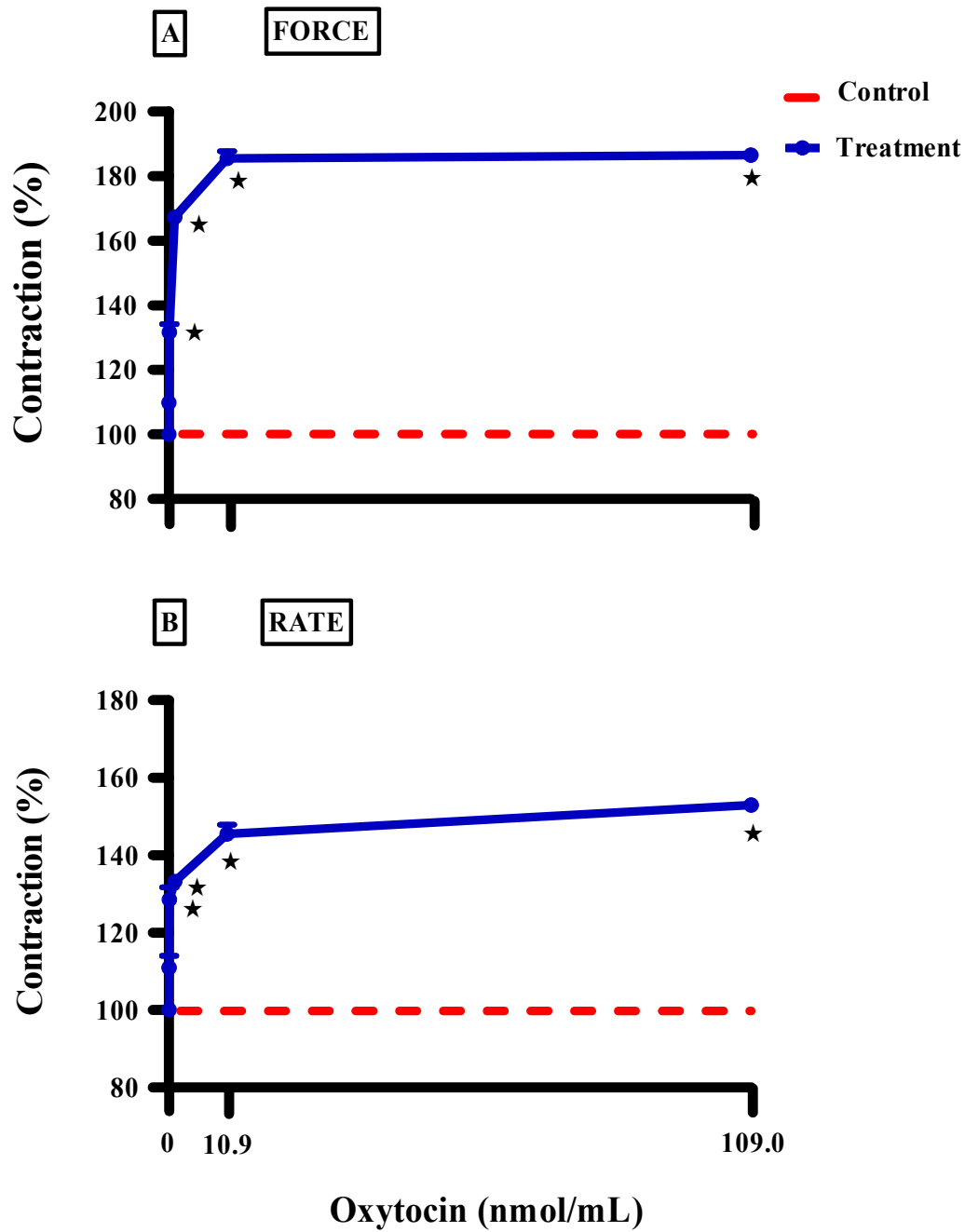
### **3.2 Concentration response curves of oxytocin and acetylcholine**

Figure 7 shows the concentration response curve of the standard drug oxytocin on uterine muscle strips. Administration of various doses of oxytocin (0.01-109.00 nmol/mL) into an organ bath evoked increases in uterine muscle contractility. Similarly, treatment of uterine muscle strips with graded concentrations of acetylcholine (0.04-4070  $\mu$ mol/mL) induced significant ( $p < 0.05$ ) increases in the uterine muscle contractility (Figure 8).

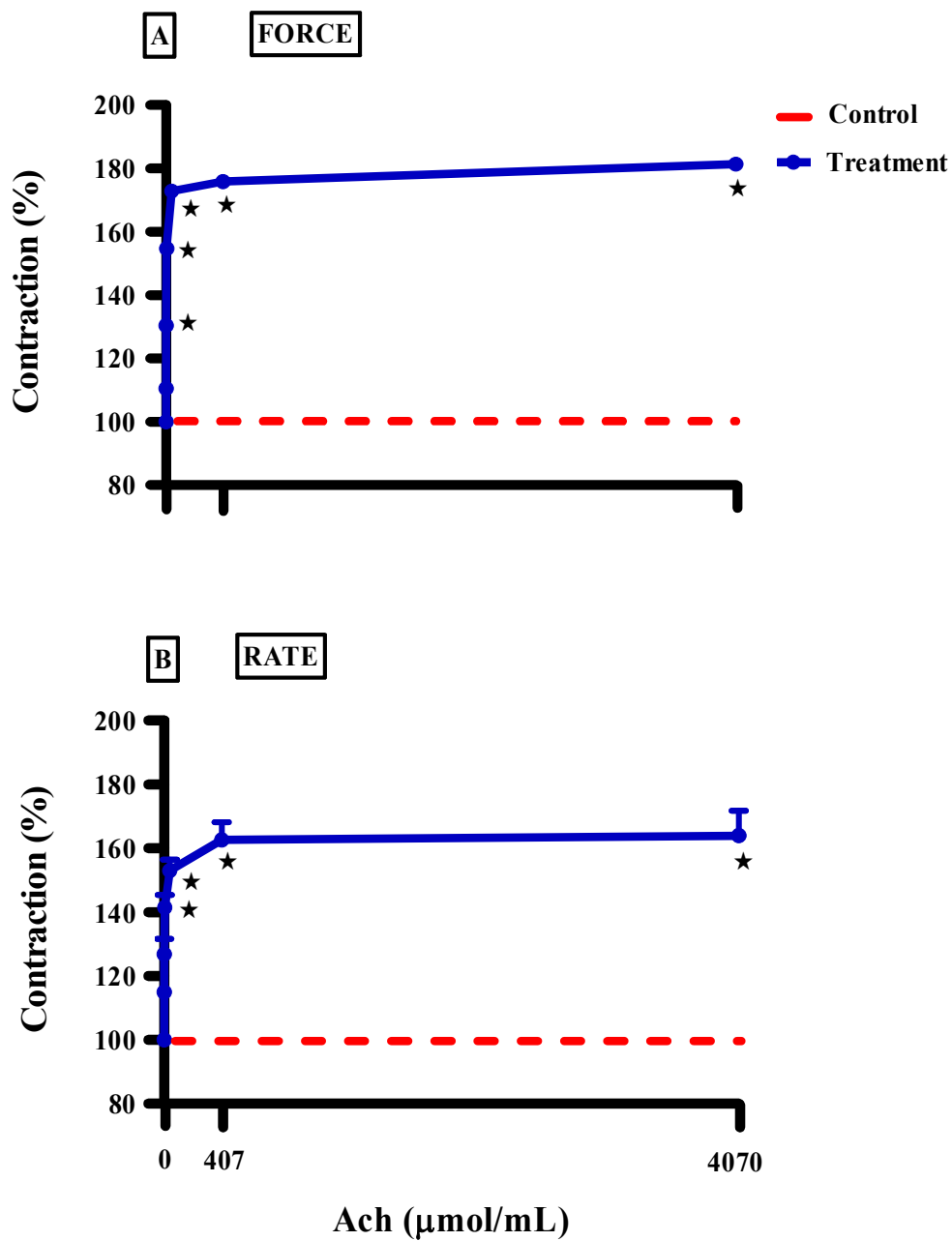
### **3.3 Uterotonic effects of *G. perpensa* methanolic root extract**

Graded concentrations of *G. perpensa* root extract (0.24-7.76 mg/mL) induced significant increases ( $p < 0.05$ ) in the force and rate of uterine muscle contractility in comparison with the control (Figure 9). Doses between 0.95 mg/mL and 3.88 mg/mL produced an increase in both the force and the rate of contractions in comparison to the control. The highest concentration (7.76 mg/mL) exhibited an increase in the force while reducing the rate of uterine muscle contraction (Figure 9). However, the concentrations of *G. perpensa* were far much higher than that of the standard drugs, oxytocin and acetylcholine (Figure 7 and 9).



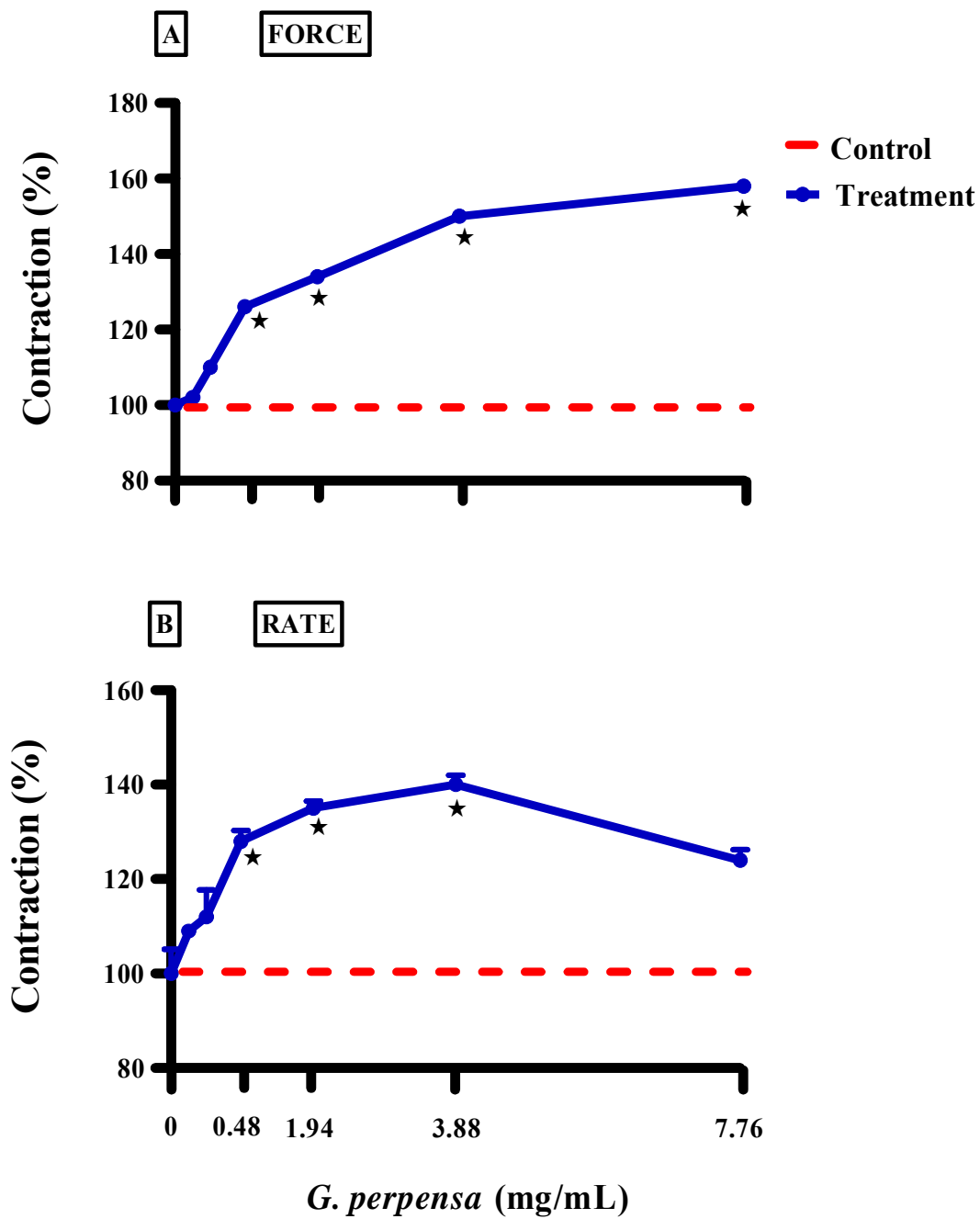


**Figure 7:** Concentration response curve of oxytocin on the force (**A**) and rate (**B**) of uterine muscle contractility. Values are expressed as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



**Figure 8:** Concentration response curve of acetylcholine (Ach) on the force (**A**) and rate (**B**) of uterine muscle contractility. Values are expressed as means  $\pm$  SEM (n=6 for each concentration).

★  $p < 0.05$  by comparison with control group.



**Figure 9:** Effects of *G. perpensa* crude extract on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.

### **3.4 Uterotonic effects of *R. tridentata* extracts**

*R. tridentata* root and leaf crude extracts as well as root-derived bioactive compounds were used to investigate the uterotonic activity. The compounds were obtained following extraction and elution with organic solvents such as DCM, methanol and acetone (Table 2).

#### **3.4.1 Crude extracts**

##### **Leaf and root methanol crude extracts**

The muscle strips were challenged with various concentrations of *R. tridentata* leaf methanol crude extract ranging between 0.24 mg/mL and 62.08 mg/mL. The lower doses (0.24-1.94 mg/mL) induced a gradual increase in both the force and rate of uterine muscle contractility to levels that did not achieve statistical significance. The data show that the statistical significance ( $p < 0.05$ ) was seen from the dose of 3.88 mg/mL and 15.50 mg/mL in the force and rate of contractions, respectively. The leaf methanol extracts increased uterine muscle contractility with the highest effects being observed at higher doses (Figure 10).

Figure 11 shows the effects on the force and rate of uterine muscle contractions following treatment with *R. tridentata* methanol root extract. Treatment of uterine muscles with various concentrations root crude extract (0.24-62.08 mg/mL) exhibited significant increases ( $p < 0.05$ ) in the force and the rate of contractility with the highest change seen at 31.0 mg/mL and 15.5 mg/mL, respectively. Conversely, the highest concentration (62.08 mg/mL) induced a reduction in the force of contractions. Similar effects were observed with oxytocin and acetylcholine, however, the concentrations of the plant extracts were much higher compared to that of the standard drugs.

#### **3.4.2 Effects of combination with oxytocin, a standard drug**

##### ***R. tridentata* root methanol crude extract and oxytocin**

Figure 12 shows the combined effects of *R. tridentata* root methanol (0.24-62.08 mg/mL) crude extract and oxytocin (1.11 nmol/mL) in comparison with *R. tridentata* root crude extract alone.

The combination of these drugs showed greater uterotonic effects than when the plant extracts are used alone.

Furthermore, *R. tridentata* root extract and oxytocin combination significantly enhanced frequency of uterine muscle contractions when compared to the treatment with *R. tridentata* root extract alone. The significant increases were observed at doses between 7.8 mg/mL to 62.0 mg/mL. *R. tridentata* root extract augmented the initial response to oxytocin. However, the highest dose (62.0 mg/mL) exerted a slight reduction in the rate of contraction. Due to small yields, the effects of *R. tridentata* leaf methanol crude extract and oxytocin and acetylcholine were not established in the current study.

### **3.4.3 Uterotonic effects of fractions**

#### ***R. tridentata* methanol and acetone root fractions**

Figure 13 shows the effects induced by treatment with roots *R. tridentata* methanol fraction. Various doses of *R. tridentata* methanol fraction (0.20-8.00 mg/mL) evoked significant increases ( $p < 0.05$ ) in the force and rate of uterine muscle contractility. However, the lower doses (0.20 and 0.50 mg/mL) showed slight increases in uterine muscle contractions which were not statistical significant in comparison with control. The dose of 4.00 mg/mL produced the maximal increase in the force of contractions while 8 mg/mL induced a greatest increase in the rate of contractions (Figure 13).

Similarly, treatment of uterine muscles with various doses (0.13-4.26 mg/mL) of acetone fraction exhibited increases in the force and rate of contractions, however, the acetone fraction was more potent when compared to methanol fraction (Figure 14). These results were comparable to the effects of standard drugs, however, the concentrations of standard drugs were much lower than that of plant extracts.

### **3.4.4 Effects of *R. tridentata* root-derived bioactive compounds**

This section describes the data obtained following treatment with bioactive compounds isolated from *R. tridentata* roots. From the methanol root crude extract, DCM and acetone fractions were

prepared and purified yielding 7 stable bioactive compounds;  $\beta$ -sitosterol, asiatic-arjunolic acid, compound 285, resveratrol glycoside and flavonoids; catechin, quercitrin and morin rhamnoside whose effects on uterine muscle contractility are described below.

### **The uterotonic effects of compound 85, asiatic-arjunolic acid, $\beta$ -sitosterol and resveratrol glycoside**

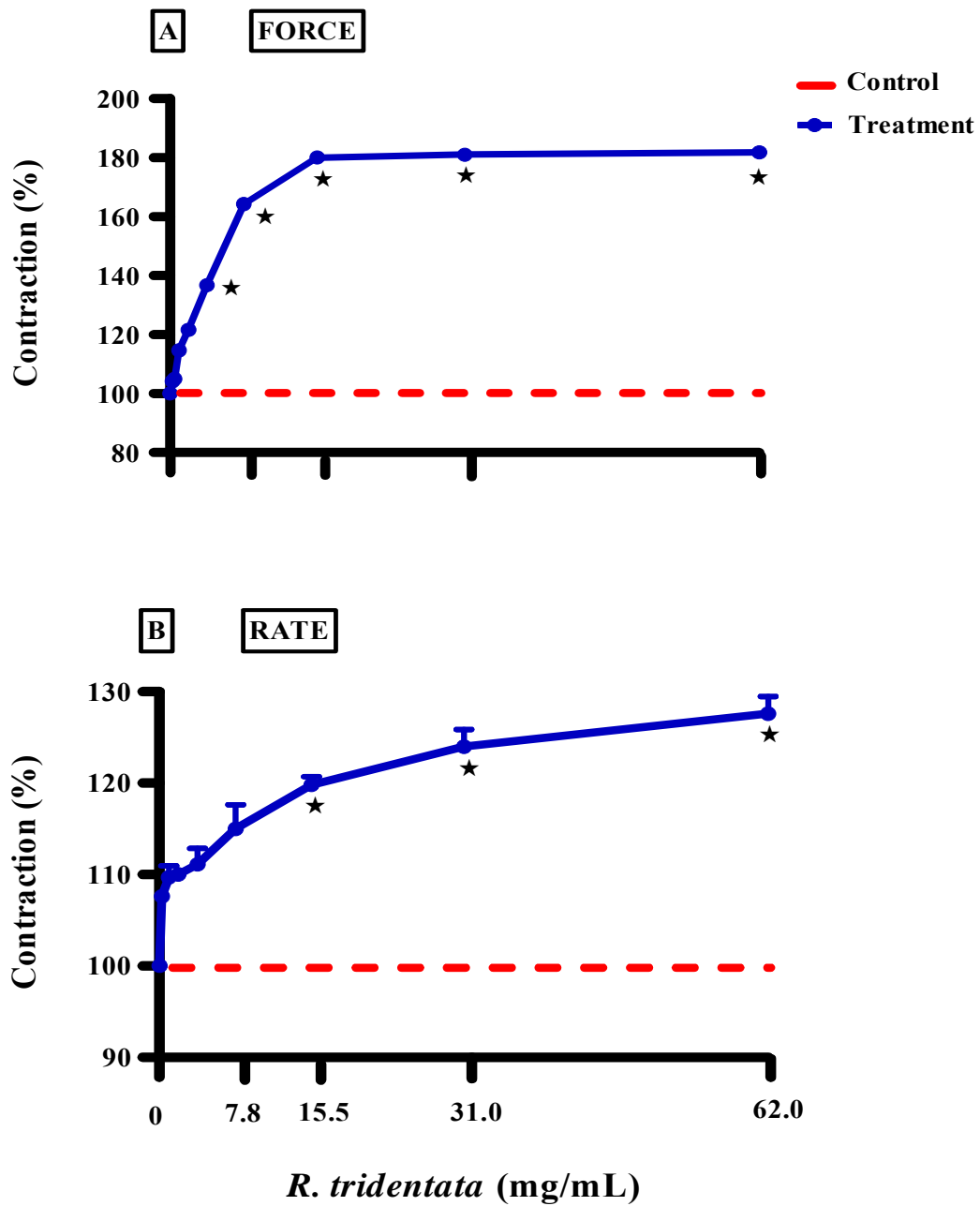
Figure 15 shows the effects of various doses (0.003-2.06 mg/mL) of compound 285 on uterine muscle contractility. Treatment of uterine muscles with this extract exhibited increases in the rate and force of contractions. A significant increase ( $p < 0.05$ ) was seen at doses 0.41 mg/mL and 2.06 mg/mL when compared to the control. Treatment with asiatic-arjunolic acid complex (0.01-6.14 mg/mL) produced an increase in the force and rate of uterine muscle contractility, however, there was no statistical significance in comparison with the control (Figure 16). The dose of 1.23  $\mu$ mol/mL produced the maximal force and rate on the uterine muscle contractility. The doses of asiatic-arjunolic acid complex were much higher than that of standard drugs, however, produced effects with no statistical significance.

Figure 17 shows the effects of graded concentrations of  $\beta$ -sitosterol (0.09-57.10  $\mu$ mol/mL) on the contractility of the uterine muscles. Administration of the drug induced increases in force and rate of uterine muscle contractions.  $\beta$ -sitosterol induced much greater effects when compared to compound 85 and asiatic-arjunolic acid complex. The dose of 11.40  $\mu$ mol/mL produced maximal rate of contraction. These results were comparable to the effects of standard drugs, although, the concentrations of these compounds were higher.

The vasorelaxant effects of resveratrol glycoside (0.37-22.80  $\mu$ mol/mL) were observed following treatment with graded concentrations of this compound. The rate and force of uterine muscle contractions were reduced with the significance ( $p < 0.05$ ) starting at 0.91 to 22.80  $\mu$ mol/mL (Figure 18).

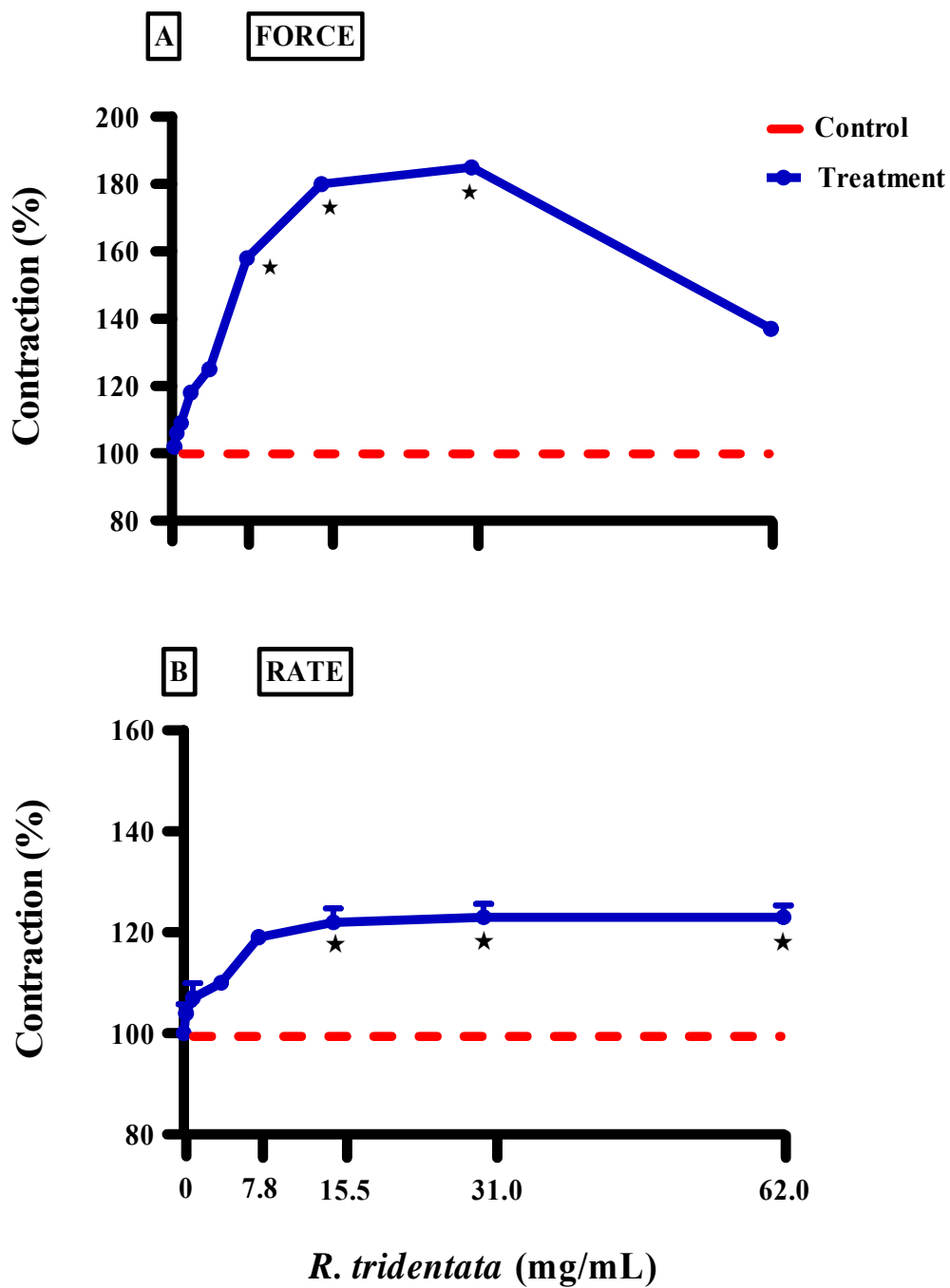
### **Effects of flavonoids; morin rhamnoside, catechin and quercitrin**

Treatment of uterine muscles with graded concentrations of morin rhamnoside (0.02-10.80  $\mu\text{mol/mL}$ ) exerted a decline in the both the rate and force of uterine muscle contractility in comparison to the control. Morin rhamnoside exhibited a significant reduction ( $p < 0.05$ ) in the force of uterine muscle contractions at 0.43  $\mu\text{mol/mL}$  to 10.80  $\mu\text{mol/mL}$ . However, the reduction in the rate of contractions was not statistical significant. The maximal inhibitory effects in both the rate and force of contractions were observed at 2.16  $\mu\text{mol/mL}$  (Figure 19). These compounds exhibited opposite effects in comparison with the standard drugs. Furthermore, administration of either catechin or quercitrin into an organ bath completely abolished the uterine muscle contractions.

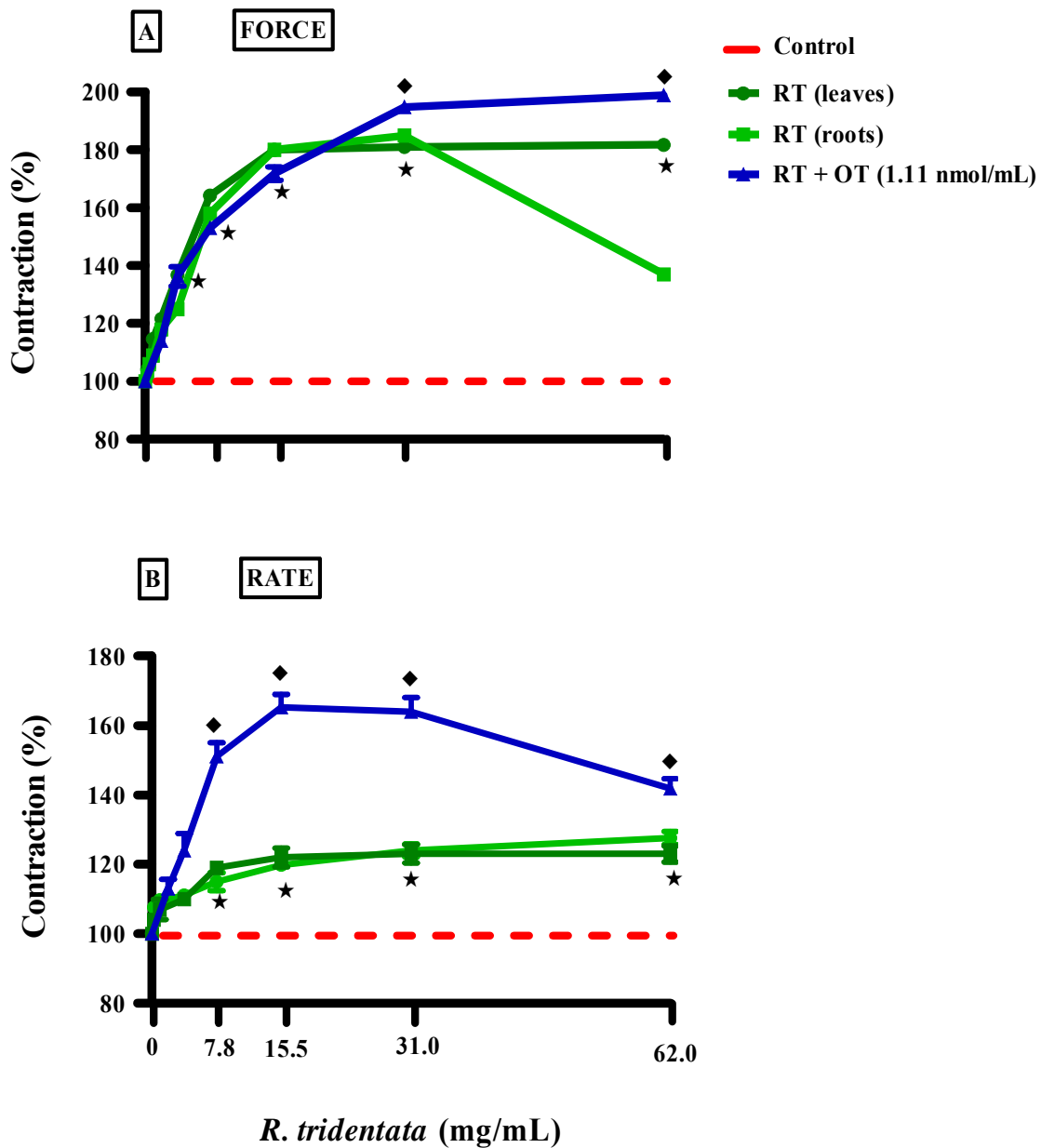


**Figure 10:** Effects of *R. tridentata* leaf crude extract on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.

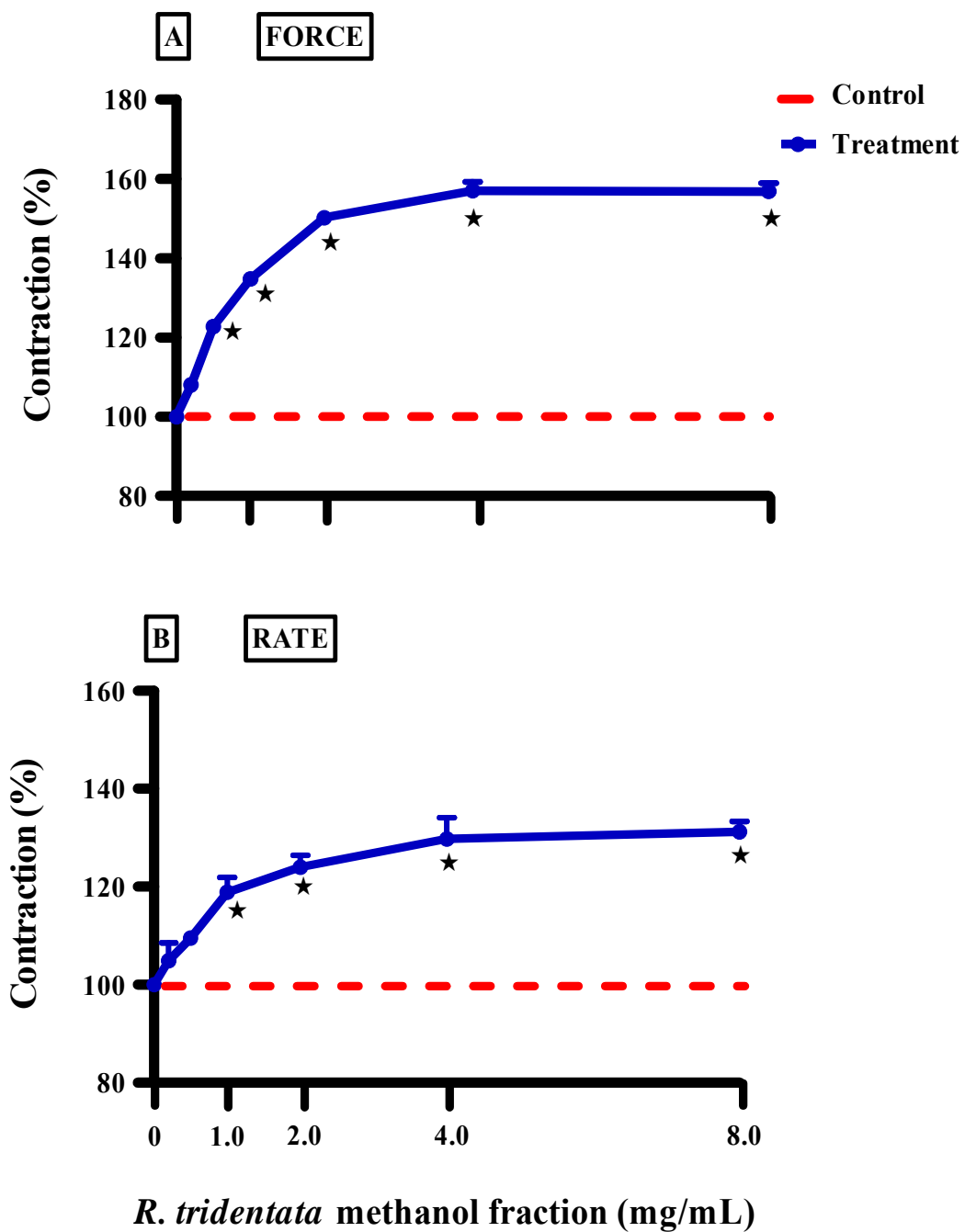




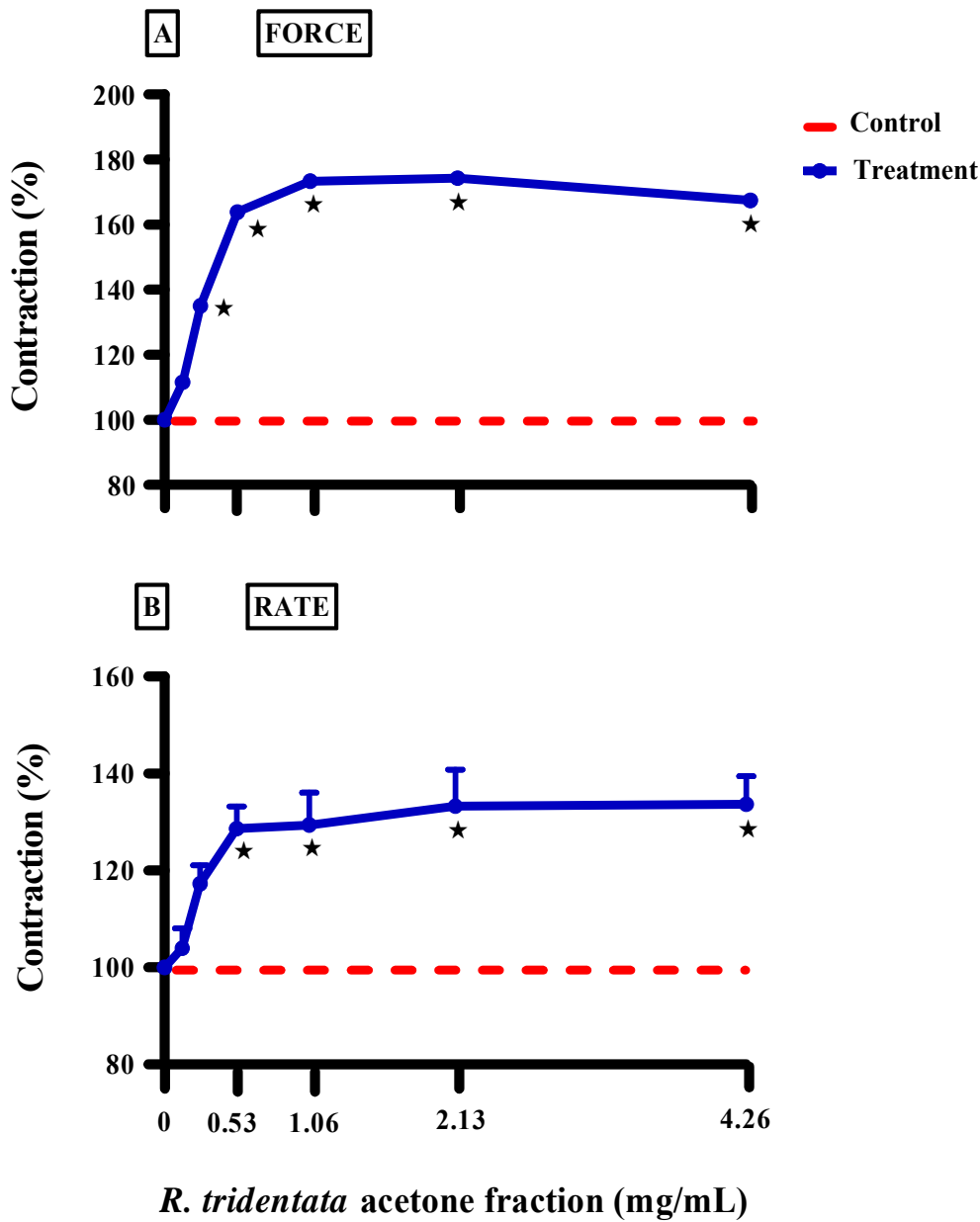
**Figure 11:** Effects of *R. tridentata* root crude extract on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



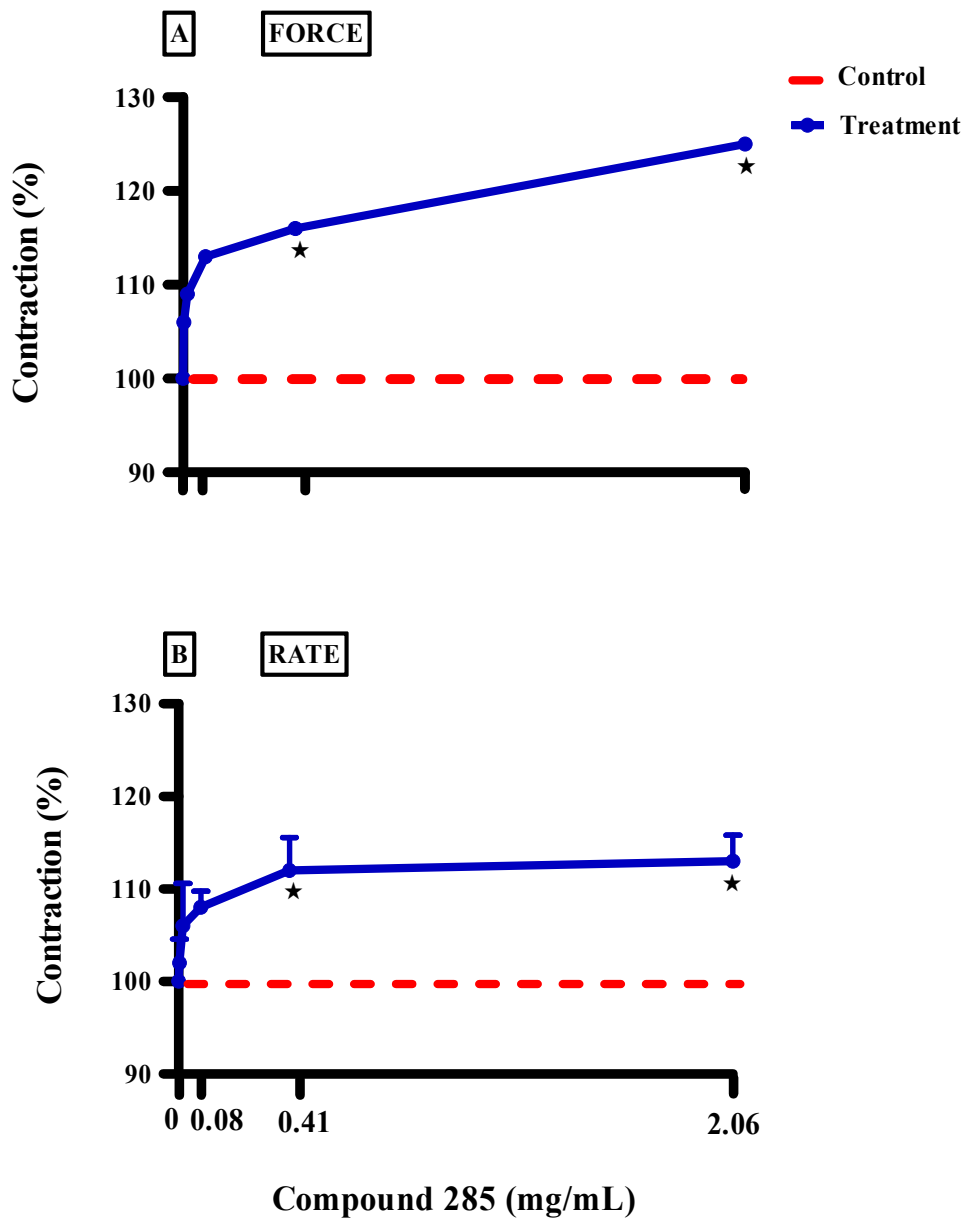
**Figure 12:** Effects of *R. tridentata* root and leaf crude extracts on the force (A) and rate (B) of uterine muscle contractility in the absence and in the presence of oxytocin (OT) (1.11 nmol/mL). Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group. ♦  $p < 0.05$  by comparison with *R. tridentata* (RT) root extract.



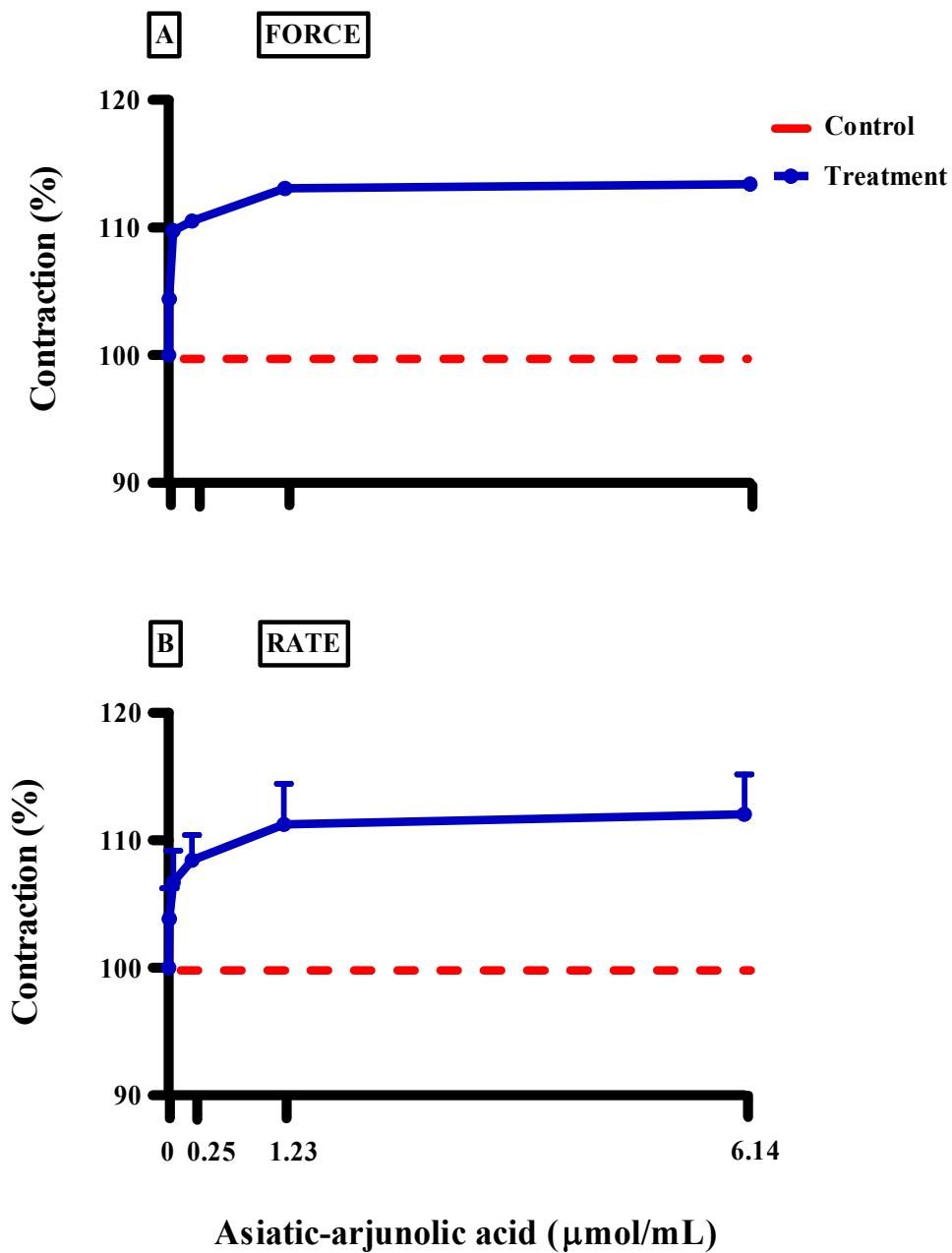
**Figure 13:** Contractile effects of *R. tridentata* root methanol fraction on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



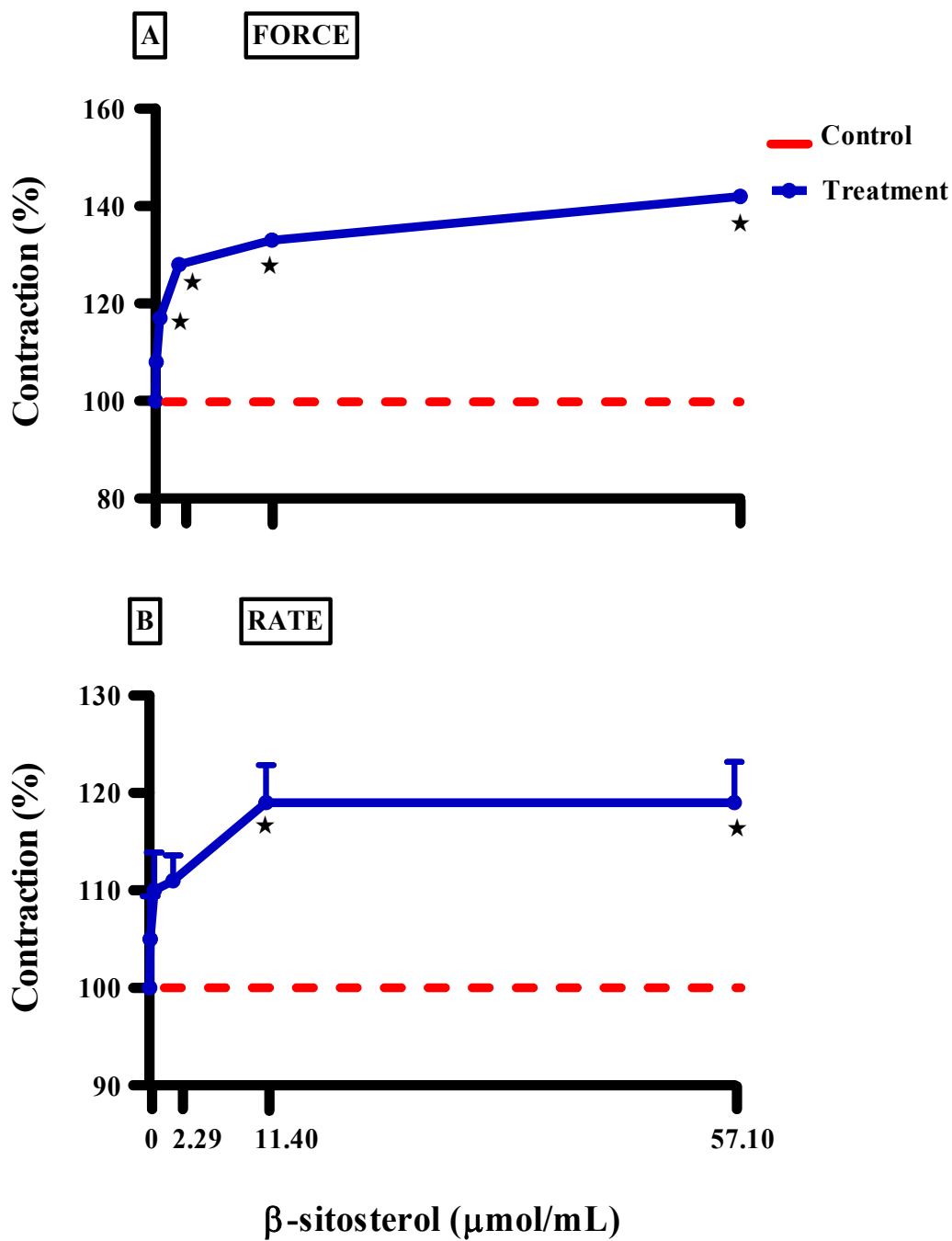
**Figure 14:** Contractile effects of *R. tridentata* root acetone fraction on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



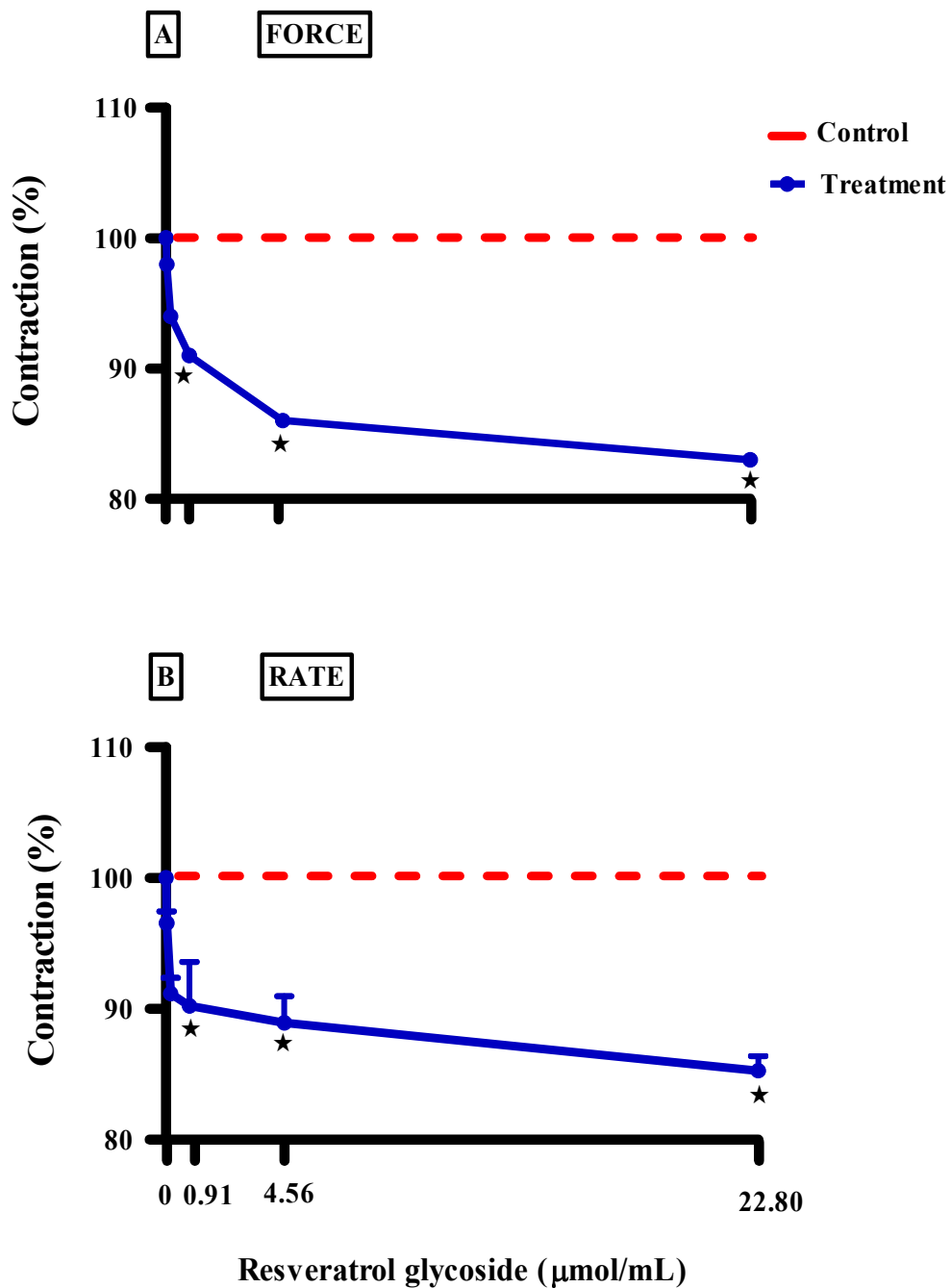
**Figure 15:** Effects of *R. tridentata* root-derived acetone compound 285 on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



**Figure 16:** Effects of *R. tridentata* root-derived asiatic-arjunolic acid on the force (A) and rate (B) of uterine muscle contractility. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★ p < 0.05 by comparison with control group.

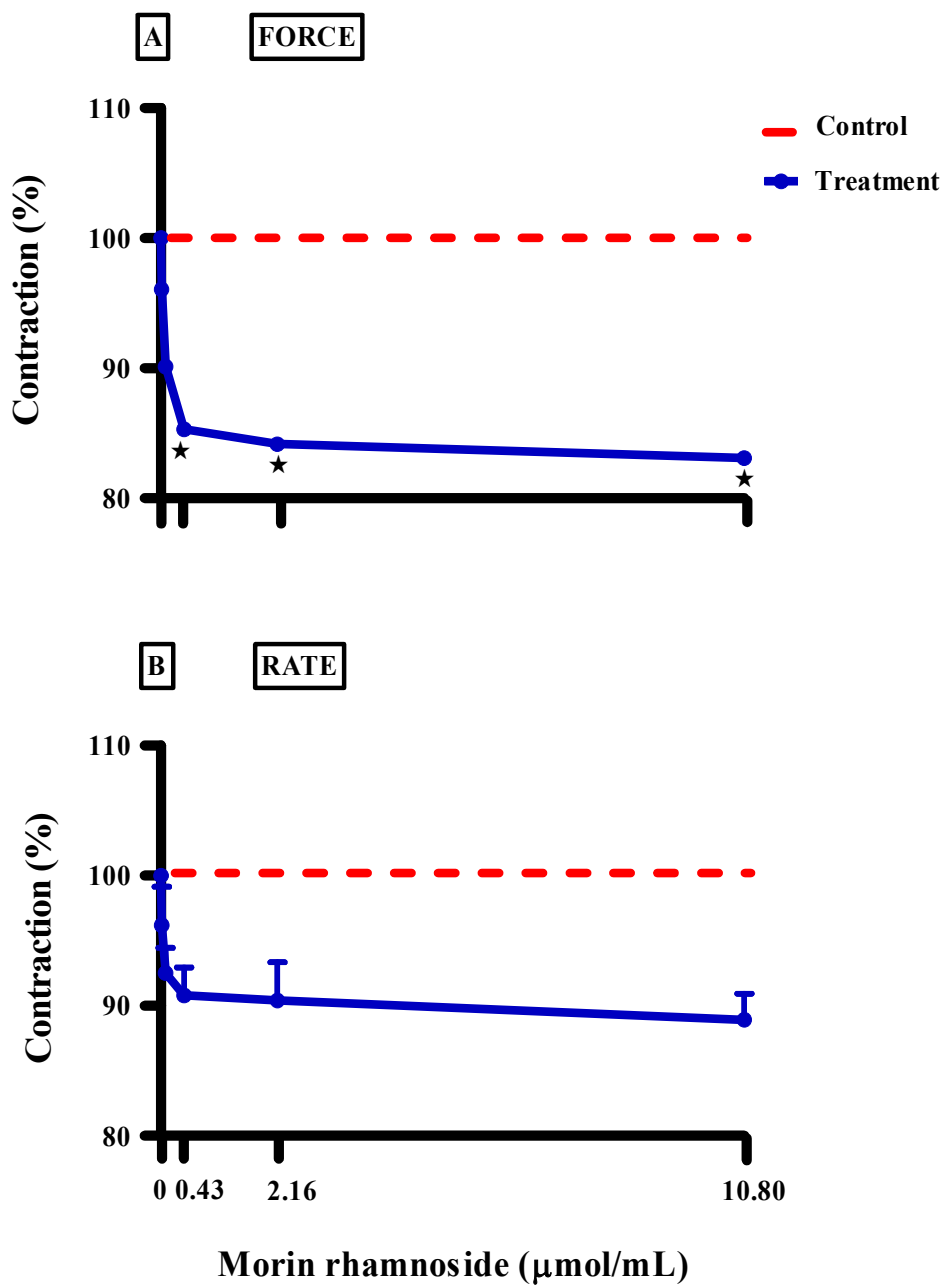


**Figure 17:** Uterotonic effects of *R. tridentata* root-derived  $\beta$ -sitosterol on the force (A) and rate (B) of uterine muscle contractions. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.



**Figure 18:** Vasorelaxant effects of *R. tridentata* root-derived resveratrol glycoside on the force (A) and rate (B) of uterine muscle contractions. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.





**Figure 19:** Vasorelaxant effects of *R. tridentata* root-derived morin rhamnoside on the force (A) and rate (B) of uterine muscle contractions. Values are presented as means  $\pm$  SEM (n=6 for each concentration). ★  $p < 0.05$  by comparison with control group.

### 3.5 Summary of results

*G. perperna* and *R. tridentata* roots and leaves were collected, extracted and eluted with various organic solvents, DCM, methanol and acetone where stable compounds were achieved. Previously validated protocols were used to achieve the results described in this study. The concentrations of plant extracts used in the present study varied according to the yields obtained following extraction. The effects of plant extracts were compared to those of the standard drugs, oxytocin and acetylcholine.

Studies on the contractile effects of *G. perperna* and *R. tridentata* extracts indicate that the leaf and root crude extracts possess uterotonic effects in experimental animals. This was observed when the uterine muscle strips were treated with various concentrations of the plant extracts. Treatment of uterine muscles *R. tridentata* root crude extracts combined with oxytocin produced higher effects in comparison with the root extract alone. Studies have also demonstrated that the methanol and acetone fractions prepared from the root *R. tridentata* root extract elicited increases in the force and rate of spontaneously contracting uterine muscles in comparison to their respective controls. The results of the plant extracts were comparable to that of standard drugs, however, the concentrations of the standard drugs were lower.

We also investigated the effects of *R. tridentata* root-derived bioactive compounds ( $\beta$ -sitosterol, compound 285, asiatic-arjunolic acid, resveratrol glycoside as well as flavonoids; morin rhamnoside, quercitrin and catechin) on uterine muscle strips. We found that  $\beta$ -sitosterol, compound 285, asiatic-arjunolic acid have a potential to increase uterine muscle contractions, however,  $\beta$ -sitosterol was the most effective compound. On the other hand, resveratrol glycoside and morin rhamnoside elicited vasolarexant effects while quercitrin and catechin completely abolished the uterine muscle contractility.

## CHAPTER 4

### DISCUSSION

The present study was designed to investigate the contractile effects of *G. perperisa* and *R. tridentata* extracts *in vitro* in an effort to establish their potential as uterotonic agents. The findings indicate that both the crude extracts and bioactive compounds of *G. perperisa* and *R. tridentata* possess uterotonic activity in experimental animals.

Studies have shown that the concentration of the bioactive compounds in plants is influenced by seasonal variation, therefore, roots and leaves of *G. perperisa* and *R. tridentata* were collected in summer (December 2012) to exclude the variation reported by Brookes and Katsoulis *et al* (Brookes and Katsoulis, 2006). The isolation techniques for the bioactive compounds were dependent on the type of compounds present in these plants based on literature evidence. The procedures used and the reproducibility of the effects of extracts indicate that stable compounds were isolated. The perfused rat uterine muscle strips have been extensively used in research laboratories to investigate the biological activity of either plant extracts and or synthetic drugs (Kamanyi *et al.*, 1992; Veale *et al.*, 1999). This protocol also assists with the elucidation of the receptor mechanisms in the uterus and has been previously used in our laboratory (Simelane *et al.*, 2012). Hence, the results that we achieved were obtained using previously validated protocols.

Previous reports indicated that crude extracts of *Clivia miniata* and *Agapanthus africanus* induce contractions in uterine muscle strips *in vitro* (Veale *et al.*, 2000). The continuous use of medicinal plants by women during pregnancy suggests that some plants may have an ability to either induce or promote foetus expulsion (Steenkamp, 2003). Hence, this has driven researchers to investigate the influence of plants on uterine muscle contractility. *G. perperisa* and *R. tridentata* are used in the preparation of *isihlambezo*, a uterotonic herbal remedy prescribed by traditional practitioners to pregnant women to hasten childbirth and prevent postpartum bleeding and hence, these plants were studied (Joseph and Mafatle, 1994; Veale *et al.*, 2000). Observations in the current study correlate with those of Brookes and Dutton who reported that

methanol extracts of *G. perpensa* root increase the contractility of uterine muscle strips however, through unknown mechanisms (Brookes and Dutton, 2007). Scientific reports indicate that *G. perpensa* contains several bioactive compounds that may be effective in the management of many gynaecological disorders (Brookes and Dutton, 2007). Literature evidence indicates that *G. perpensa* crude extracts and isolated bioactive compounds such as *Z-venusol*, 1,1'-biphenyl-4,4'-diacetic acid, *Z*-lespedezic acid methyl ester, ellagic acid lactone, 3,3',4'-trimethyl ellagic acid acetone, *Z*-methyl lespedezate possess many therapeutic properties including placenta expulsion, wound healing and milk ejection (Brookes and Dutton, 2007; Nkomo *et al.*, 2010; Simelane *et al.*, 2012). *G. perpensa* has been previously shown to possess antihemorrhagic, antimutagenic and anticarcinogenic properties thus indicating that this plant apart from exerting uterotonic effects, plays a protective role to both maternal and foetal tissues (Varga and Veale, 1997; Azeez *et al.*, 2013). Hence, these studies suggest that *G. perpensa* has a potential to provide cheap and easily accessible treatment which would prevent or alleviate an array of complications during pregnancy. Khan *et al* reported that *G. perpensa*-derived *venusol* enhances spontaneous contractions of uterine muscles indicating that plants contain many compounds with beneficial effects. Simultaneous administration of *G. perpensa* increased the force and rate of contractility while some studies have only observed these effects after washing (Khan *et al.*, 2004). We suggest that the discrepancy may be attributed in part to the extracts preparation and hence, bioactive compounds present. Observations in this study complement those of Katsoulis *et al* and Simelane *et al* who revealed that decoctions of *R. tridentata* and *G. perpensa* exhibit uterotonic effects in rats (Katsoulis *et al.*, 2000; Simelane *et al.*, 2012). Simelane *et al* also documented that *G. perpensa* promotes milk letdown through induction of myoepithelial contraction and inhibition of the enzyme acetylcholinesterase activity. Even though the exact mechanisms through which *G. perpensa* exerts uterotonic effects are not fully understood, we speculate that this effect may be mediated through the opening of  $\text{Ca}^{2+}$  channels, the voltage gated channels (VGC) and receptor operated channel (ROC) since these  $\text{Ca}^{2+}$  channels are responsible for the influx of  $\text{Ca}^{2+}$  ion from the extracellular fluid to cytosol thus triggering contractions (Jenkin and Young, 2004). Furthermore, the reported antioxidant activity of *G. perpensa* may enhance the function of smooth muscle through improving oxidative status since reactive oxygen species contribute to the failure of smooth muscle contractility (Simelane *et al.*, 2010).

We have also shown that *R. tridentata* roots contain uterotonic bioactive compounds such as  $\beta$ -sitosterol, compound 285 and asiatic-arjunic acid which may play beneficial roles by promoting expulsion of the foetus. On the other hand, tocolytic compounds found in *R. tridentata* include resveratrol glycoside and flavonoids such as morin rhamnoside, quercitrin and catechin may reduce risks for preterm labour, thus stabilizing pregnancy. Katsoulis *et al.*, demonstrated that the uterotonic effects of *R. tridentata* are mediated via binding to muscarinic receptors and through the synthesis of cyclooxygenase metabolites (Katsoulis *et al.*, 2000).

During the last trimester, a number of oxytocic receptors used by this uterotonic are up-regulated by steroids, hence, increased uterine contractility is expected. Studies have documented that the concentration of uterotonic receptors such as oxytocin receptors is low between 13 and 17 weeks, however, a 12 fold increase was observed at 34 to 41 weeks. This shows that the receptors responsible for the contraction of the uterus are highly up-regulated during the last trimester (Larcher *et al.*, 1995). The tocolytic compounds derived from *R. tridentata* may require different receptors to exert their inhibitory effects. Receptors such as those of progesterone are responsible for the inhibition of the myometrial contraction during pregnancy, however, this hormone and the associated receptors are down-regulated at term. Hence, we speculate that the tocolytic effects of *R. tridentata*-derived bioactive compounds may be mediated in part via binding to inhibitory receptors such as those of progesterone, thus stabilizing pregnancy.

Despite of the differences in doses, medicinal plants have been shown to be less toxic and have fewer side effects in comparison with the conventional drugs and to can be used in combination with the synthetic drugs, thus promoting efficacy of the standard drugs. Co-administration of *R. tridentata* root crude extract with oxytocin induced an additive effect when compared to the plant extract alone, however the mechanisms of action cannot be established by data in the current study. We speculate that these effects could be mediated via  $\text{Ca}^{2+}$  influx from extracellular fluid or through cyclooxygenase metabolites production as previously explained by Katsoulis *et al.* (Katsoulis *et al.*, 2000). This effect may be beneficial in conditions such as post-term labour. Hence, to support the current findings, there is a need to explore the mechanisms of action in these extracts mediate their effects.

In addition, this study investigated the effects of  $\beta$ -sitosterol, compound 285, asiatic-arjunic acid, resveratrol glycoside, morin rhamnoside, quercitrin and catechin which are the bioactive

compounds derived from *R. tridentata* root extract. Treatment of uterine muscles with  $\beta$ -sitosterol, compound 285 and asiatic-arjunic acid evoked increases in the rate and force of contractions through unknown mechanisms. Katsoulis *et al* demonstrated that *R. tridentata* contains imberbic acid and  $\beta$ -sitosterol which mostly account for the uterotonic activity of this plant. Katsoulis *et al* identified many bioactive compounds in *Rhoicissus tridentata* including epigallocatechin, gallic acid hydrate, mollisacacidin, epicatechin, oleanolic acid, prostacyandin B3 and B4, as well as garlic acid. This plant is commonly used in the preparation of *isihlambezo*, a herbal remedy used by pregnancy women to prepare the uterus for foetus expulsion. Hence, these bioactive compounds are beneficial in promoting foetal good health and childbirth. The proanthocyanidins found in *Rhoicissus tridentata* are uterotonic agents and their concentration vary according to the geographical source of the plants (Brookes and Katsoulis, 2006).

Promprom *et al* evidenced the uterotonic effects of plant-derived  $\beta$ -sitosterol and deduced that this effects was due to  $\text{Ca}^{2+}$  influx and through activation of myosin light kinase, an enzyme responsible for the phosphorylation of the myosin heads and thus triggering contractions (Promprom *et al.*, 2010). We postulate that this could be one of the possible mechanisms by which root *R. tridentata*-derived  $\beta$ -sitosterol, compound 285 and asiatic-arjunolic acid accelerate myometrial contractions.

These results were comparable to those of oxytocin and acetylcholine, the standard drugs which promote parturition by triggering myometrial contractions through activating the specific membrane-bound receptors. Hormonal and neurotransmitter stimulation induces the  $\text{Na}^+$  influx resulting in membrane depolarization which in turn excites the release of  $\text{Ca}^{2+}$  from sarcoplasmic reticulum (SR) and consequently uterine muscle contraction occurs (Flandermeyer *et al.*, 2010). Our data have also shown that root extracts of *R. tridentata* contain resveratrol glycoside, a compound which exerted inhibitory effects in the rate and force of uterine muscle contractility through undefined mechanisms. Reports by Novakovic *et al* indicated that resveratrol inhibited the contractility of arteries and veins via exciting voltage-gated  $\text{K}^+$  channels and  $\text{Ca}^{2+}$ -sensitive  $\text{K}^+$  channels (Novakovic *et al.*, 2013). Therefore, based on these observations, we speculate that *R. tridentata*-derived resveratrol glycoside causes relaxant effects via activating the voltage-gated  $\text{K}^+$  channels, increasing levels of cAMP or by promoting the synthesis of progesterone,

leading to the inhibition of uterine muscle contractions, thus stabilizing pregnancy in case of preterm term labour (Bradshaw and Bradshaw, 2011). However, these mechanisms remain elusive. Similar effects were observed during treatment with morin rhamnoside whereas other flavonoids, quercitrin and catechin completely abolished the contractions. Literature reveals that synthetic quercitrin exhibited stimulatory as well as long-lasting inhibitory effects in experimental animals (Ojewole and Chiwororo, 2010). The inhibitory effects of quercitrin and catechin may be attributed to toxicity of these compounds, inhibition of  $\text{Ca}^{2+}$ -sensitizing mechanisms or inhibition of cyclic AMP phosphodiesterase as postulated previously (Beretz *et al.*, 1980). Our results suggest that *G. perpersa* and *R. tridentata* root and leaf extract can be used to induce labour and in the management of other gynaecological complications.

## CHAPTER 5

### CONCLUSIONS

#### 5.0 Conclusions

The data suggests that *G. perpersa* and *R. tridentata* root and leaf crude extracts increase the force and the rate of uterine muscle contractions. Furthermore, these results suggest that *R. tridentata* root-derived bioactive ingredients possess uterotonic effects. However, *R. tridentata* also contains tocolytic compounds. Therefore, these plants have a potential to promote childbirth preventing postpartum haemorrhage and avert preterm labour and other gynaecological complications. However, more studies are essential to establish the mechanisms of action.

#### 5.1 Limitations and recommendations for future studies

The limitations of the current study are that the mechanisms of action of the plant extracts were not established. Therefore, there is a necessity to investigate the possible mechanisms through which *G. perpersa* and *R. tridentata* extracts exert their effects. To establish the possible mechanisms, future studies will use various receptor blockers such as atosiban, an oxytocin receptor antagonist, atropine which is a muscarinic receptor antagonist as well as indomethacin, cyclooxygenase inhibitor. Future studies will investigate the effects plant extracts on ion exchange in the cells and the toxicity studies of these plant extracts will be performed.



## CHAPTER 6

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## CHAPTER 7

### APPENDICES

#### APPENDIX I: Ethical clearance certificate A

**UNIVERSITY OF  
KWAZULU-NATAL**  
INYUVESI  
**YAKWAZULU-NATALI**  
**Research Office**  
**Animal Ethics Research Committee**  
Govan Mbeki Centre, Westville Campus,  
University Road, Chiltern Hills, Westville, 3629, South Africa  
Telephone 27 (031) 260-2273/35 Fax (031) 260-2384  
Email: [animalethics@ukzn.ac.za](mailto:animalethics@ukzn.ac.za)

15 March 2013

Reference: 087/13/Animal

Miss S Dube  
School of Laboratory Medicine  
and Medical Sciences  
University of KwaZulu-Natal  
WESTVILLE Campus


Dear Miss Dube

**Ethical Approval of Research Projects on Animals**

I have pleasure in informing you that the Animal Ethics Sub-committee of the University Ethics Committee has granted ethical approval for 2013 on the following project:


**"Contractile effects of *Gunnera perpensa*, *Agapanthus africanus*, *Clivia miniata*, *Typha capensis*, *Rhoicissus tridentate* and *Pentania prunelloides* bioactive extracts on isolated rat uterine, cardiac and vascular muscles."**

Yours sincerely






  
**Professor Theresa HT Coetzer**  
**Chairperson: Animal Ethics Sub-committee**

Cc Registrar – Prof. J Meyerowitz  
Research Office – Dr N Singh  
Supervisor, Prof. C Musabayane  
Head of School – Prof. W Daniels  
BRU, Dr S Singh

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Founding Campuses:

-  Edgewood
-  Howard College
-  Medical School
-  Pietermaritzburg
-  Westville

## APPENDIX II: Ethical clearance certificate B



23 December 2013

Reference: 032/14/Animal

Miss S Dube  
Discipline of Physiology  
School of Laboratory Medicine &  
Medical Sciences  
WESTVILLE Campus

Dear Miss Dube

### RENEWAL: Ethical Approval of Research Projects on Animals

I have pleasure in informing you that the Animal Research Ethics Committee has granted ethical approval for **2014** on the following project:

**"Contractile effects of *Gunnera perpensa*, *Agapanthus africanus*, *Clivia Miniata*, *Rhoicissus tridentate* and *Typha capensis* bioactive extracts on isolated rat uterine, cardiac and vascular muscles."**

Yours sincerely

**Professor Theresa HT Coetzer**  
**Chairperson: Animal Research Ethics Committee**

Cc Registrar – Prof. J Meyerowitz  
Research Office – Dr N Singh  
Supervisor – Prof. C Musabayane  
Head of School – Prof. W Daniels  
BRU – Dr S Singh

#### Animal Ethics Committee Professor Theresa HT Coetzer (Chair)

Postal Address: Room 105, John Bews Building, Private Bag X01, Pietermaritzburg, 3201, South Africa  
Telephone: +27 (0)33 260 5463/35 Facsimile: +27 (0)33 260 5105 Email: [animalethics@ukzn.ac.za](mailto:animalethics@ukzn.ac.za) Website: [www.ukzn.ac.za](http://www.ukzn.ac.za)  
Founding Campuses: ■ Edgewood ■ Howard College ■ Medical School ■ Pietermaritzburg ■ Westville



INSPIRING GREATNESS

### APPENDIX III: ORAL PRESENTATIONS

Presentation at the Physiological Society of Southern Africa, 41<sup>st</sup> Congress hosted by the University of Limpopo in September 2013

#### ABSTRACT SUBMISSION

**TITLE: EVALUATION OF THE MECHANISMS OF THE HYPOTENSIVE EFFECTS OF *SYZYGIUM AROMATICUM*- DERIVED OLEANOLIC ACID IN EXPERIMENTAL ANIMAL PARADIGMS**

Author/s: **Sinenkosi Dube and Cephas T Musabayane**

Affiliation/s: **University of KwaZulu-Natal**

**Text:**

We have reported that oleanolic acid (OA) decreases blood pressure in experimental animals <sup>[1]</sup>. Accordingly, this study was designed to investigate the mechanisms of the hypotensive effects of OA in experimental animal paradigms. To establish whether the hypotensive effects of OA were mediated via altering renal fluid and electrolyte handling, mean arterial pressure (MAP) as well as renal function were compared in groups of weanling Dahl salt-sensitive (DSS) rats, the genetic model of hypertension and normotensive Wistar rats treated twice every third day for eight weeks with various doses of OA (30, 60, 120 mg/kg, p.o.). Control animals were administered deionized water / dimethyl sulfoxide (DMSO) (3ml/kg). The effects of OA on oxidative stress in the liver, heart and kidney tissues of DSS rats were investigated by monitoring malondialdehyde (MDA) levels, the lipid peroxidation biomarker and activity of antioxidant enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GPx) in these tissues, as sustained oxidative stress leads to organ dysfunction <sup>[2]</sup>. Contractile effects of OA on the heart were investigated *in vitro* using isolated right atrial muscles of normotensive Wistar rats in the presence and absence of appropriate antagonists. DSS-untreated rats progressively developed high blood pressure from week 3 until the end of the experiments perhaps due to Na<sup>+</sup> retention observed. OA treatment significantly reduced the mean arterial pressure in DSS rats with a concomitant increase in Na<sup>+</sup> excretion. Significant increase of MDA and decreases of SOD and GPx were found in hepatic, cardiac and renal tissues of untreated DSS rats as compared to normotensive control animals. Treatment of diabetic rats with OA significantly reduced the MDA in all tissues and increased the activities of SOD and GPx in the liver and kidney compared to untreated DSS group. *In vitro studies* further show that OA increased the contractility of the heart in a dose-dependent manner; however, the OA-induced effects were abolished by pretreatment with propranolol, suggesting that



these effects are perhaps mediated by activation of the beta-adrenergic receptors. The observations suggest that the OA-induced natriuresis and improving oxidative stress contributes to the triterpene's hypotensive effects in DSS rats.

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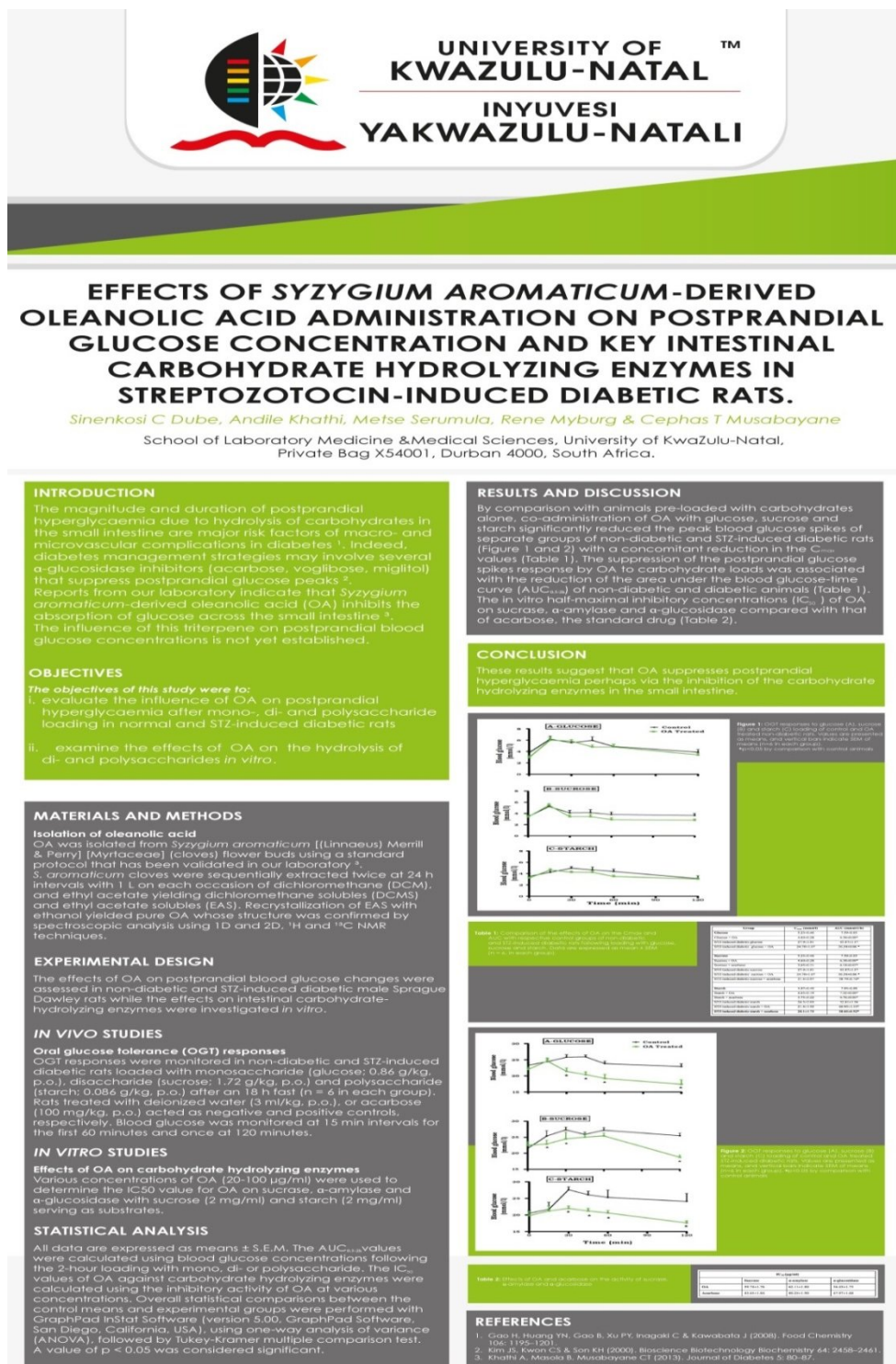
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Oral presentation at the Physiological Society of Southern Africa, 42<sup>st</sup> Congress hosted by the University of KwaZulu in September 2014

ABSTRACT SUBMISSION	
<b>Title:</b>	<b>CONTRACTILE EFFECTS OF <i>RHOICISSUS TRIDENTATA</i> BIOACTIVE EXTRACTS ON ISOLATED RAT UTERINE MUSCLES</b>
<b>Author/s:</b>	<b><sup>1</sup>Sinenkosi C Dube, <sup>2</sup>Bongiwe Madlala, <sup>2</sup>Fanie R Van Heerden &amp; <sup>1</sup>Cephas T Musabayane</b>
<b>Affiliations/s</b>	<b>Schools of <sup>1</sup>Laboratory Medicine and Medical Sciences and <sup>2</sup>Chemistry and Physics, University of KwaZulu-Natal, Private Bag X54001, Durban 4000, South Africa</b>
<p>Reports indicate that <i>Rhoicissus tridentata</i> has been widely used by women to alleviate gynaecological complications including post-term labour and postpartum bleeding. Literature evidence suggests that <i>R. tridentata</i> possesses uterotonic effects which are mediated in part via hyperstimulation of the uterus. However, further studies are required to investigate the uterotonic compounds in <i>R. tridentata</i>. Accordingly, this study was designed to investigate the contractile effects of <i>R. tridentata</i> bioactive extracts on isolated rat uterine muscles. Oxytotic activity of the plant extracts was compared with oxytocin, a uterine muscle stimulant. Crude extracts (methanol and acetone) and bioactive compounds (beta sitosterol, resveratrol and asiatic-arjunolic acid) were obtained from the Chemistry department, Pietermaritzburg, University of KwaZulu Natal. Uterotonic activity of all extracts was investigated using uterine muscle strips isolated from female Sprague-Dawley rats oestrogenized with stilboestrol (12 mg/kg) 24 h prior to the experiment. Following 30 minutes equilibration, uterine strips were challenged with plants extracts in the presence or absence of oxytocin. Like oxytocin, <i>R. tridentata</i> crude extracts (0-64 mg/ml) exerted an increase in the force and rate of contraction mediated, however, through unidentified mechanisms. The combination of <i>R. tridentata</i> crude extract and oxytocin had a synergistic effect which may be beneficial in childbirth. Acetone fraction (0-4.26 mg/ml) derived from <i>R. tridentata</i> methanolic extract also amplified the uterine muscle contractions. Resveratrol exerted an inhibitory effect on the uterine muscle contractility. Interestingly, beta sitosterol and asiatic-arjunolic acid were found to be uterotonic agents. The findings suggest that <i>R. tridentata</i> contain uterotonic compounds which may be beneficial in childbirth.</p>	

## APPENDIX IV: POSTER PRESENTATIONS

Presentation at the Society for Endocrinology BES meeting held at the United Kingdom, Harrogate in March 2013 (Endocrine Abstract: Volume 31, P200)



## Chemical Constituents from *Rhoicissus tridentata*: Structural and Activity Studies.

Bongiwe P. Mshengu<sup>1,\*</sup>, Sinenkosi C. Dube<sup>2</sup>, Cephas T. Musabayane<sup>2</sup>, and Fanie R. van Heerden<sup>1</sup>  
<sup>1</sup>School of Chemistry (Pietermaritzburg Campus) and <sup>2</sup>School of Laboratory Medicine & Medical Sciences (Westville Campus)  
 University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa



### INTRODUCTION AND AIMS

The use of medicinal plants during pregnancy, birth and post-partum care is well documented throughout the world.<sup>1</sup> In South Africa, approximately 80% of pregnant women from the Zulu and Xhosa populations uses "Isihlambezo" (a herbal decoction prepared from ca. four oxytocic plants and taken orally during the last trimester of pregnancy).

*Rhoicissus tridentata* (L.f.) Wild & Drumm. subsp. *cuneifolia* (Vitaceae) is one of the six most frequently-cited species used in the preparation of "Isihlambezo". *R. tridentata* has also been reported to prevent miscarriage and diarrhoea. Previous biological tests on the aqueous extracts from the root and lignotubers showed notable *in vitro* activity on isolated rat uterine muscle tissue. The chemistry of the methanol extract of the roots of *R. tridentata* has been studied previously and the components of the most active fraction were reported to be proanthocyanidin monomers and dimers.<sup>2</sup>

- Herein we report further isolation of chemical constituents from the methanol extract from the root of *R. tridentata*.
- In addition, the *in vitro* uterotonic activity of these compounds has been evaluated.



Fig.1. Leaves and Roots of *R. tridentata*

### RESULTS AND DISCUSSION

#### Isolated Compounds

From the polar fractions (acetone and MeOH): catechin (1), quercetin (2), morin 3-O- $\alpha$ -L-rhamnopyranoside (3), *trans*-resveratrol glucoside (4), and an inseparable mixture of arjunolic acid (5) and asiatic acid (6) were isolated.  $\beta$ -Sitosterol (7) was obtained as the major compound from DCM fractions. (Fig.2) The identity of compounds 1 - 7 was confirmed by comparison of their NMR data, high-resolution mass spectra, and specific rotation with authentic samples and/or literature values.<sup>2-7</sup>

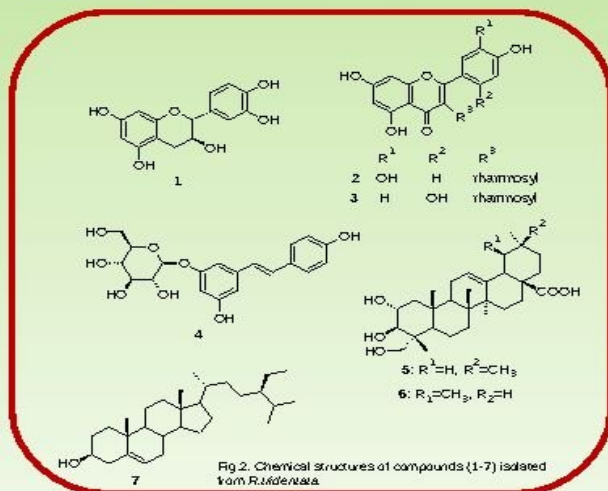


Fig.2. Chemical structures of compounds (1-7) isolated from *R. tridentata*

#### Biological activity:

The effect of *R. tridentata* crude extracts (DCM and MeOH) on the rate and force of contractions of isolated uterine muscle was evaluated. All isolated compounds were also assayed.

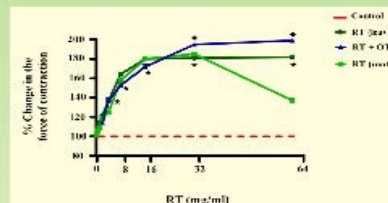


Fig.3. Effect of *R. tridentata* (RT) and oxytocin (OT) on uterine smooth muscle force of contraction. Values are represented as mean  $\pm$  SEM ( $n = 6$  for each concentration). \* $p < 0.05$  by comparison of all treated groups with the control.  $\Delta p < 0.05$  by comparison of RT with combined RT and OT.

- The MeOH extracts from both the root and leave extracts increased both the rate and the force of contractions of the isolated rat uterine muscle (Fig. 3).
- The mixture of arjunolic acid (5) and asiatic acid (6), and  $\beta$ -sitosterol (7) stimulated the direct contractile response of the uterus.
- In contrast, *trans*-resveratrol glucoside (4) and morin rhamnoside (3) decreased the rate and the force of the contractions of the uterine muscle.
- Catechin (1) and quercetin (2) completely inhibit

### CONCLUSIONS

- Compounds 1-6 are reported for the first time from *R. tridentata*.
- The uterotonic activity of arjunolic acid (5) and asiatic acid (6) is reported for the first time.
- The relaxation effect observed for *trans*-resveratrol glucoside is similar to that reported for *trans*-resveratrol.
- $\beta$ -sitosterol (7) showed the highest activity.

### ACKNOWLEDGEMENTS

We are grateful to the National Research Foundation (NRF, RSA) and the University of KwaZulu-Natal for financial support.

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