European Journal of Medicinal Plants

24(2): 1-12, 2018; Article no.EJMP.40934
ISSN: 2231-0894, NLM ID: 101583475

Uterotonic Potential of Selected Plants Used by Ugandan Local Communities in the Treatment of Malaria

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Authors' contributions

This work was carried out in collaboration between all authors. Author EK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors EK and AL carried out the laboratory experiments, managed the analyses of the study and critical reviews. All authors managed the literature searches, read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2018/40934
Editor(s):
(1) Dr. Prem K. Ramasamy, Department of Biochemistry, Brandeis University, Waltham, MA, USA.
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(1) Abdullahi A. Imam, Bayero University, Nigeria.
(2) Anonymous, Gujarat University, India.
Complete Peer review History: http://www.sciencedomain.org/review-history/25200

Original Research Article

Received 22nd March 2018
Accepted 24th May 2018
Published 20th June 2018

ABSTRACT

Malaria is a major cause of death among pregnant women and children under the age of five in Uganda. It is the leading cause of anemia among pregnant women and low birth weight in infants [1]. Majority of the rural population rely on herbs for treatment of various diseases. The leaf extracts of various plants including *Bothlioclines longipes, Vernonia amygdalina, Rhus natalensis* and *Maesa lanceolata* are used to treat various diseases including malaria in Uganda. The local communities prefer herbal preparations from the leaves of the plants. Extracts from the leaves of the plants were proven efficacious against *P. falciparum* in earlier studies *in vitro*. This study was

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carried out to investigate the effect of Diethyl ether and methanol extracts on the contractility of an isolated rabbit uterus and also to quantitatively analyze for iron and zinc in the leaves of these plants. The diethyl ether extracts of *V. amygdalina, M. lanceolata* and *R. natalensis* at a concentration of 0.4 mg/ml and 0.8 mg/ml exhibited contractility amplitudes of 26.0 mm, 21.5 mm and 27.5 mm and 22.5±1.10 mm, 15.0±1.78 mm and 24.5 ±0.99 mm respectively. The methanol extracts were 25.5±0.70 mm, 23±1.34 mm, 22±1.01 mm and 37.8±1.26 mm, 16.7±2.01 mm, 24.3±0.06 mm respectively, while oxytocin showed 22.8±0.57 mm and 31.0±0.36 mm respectively. The plant leaves were found to have high levels of iron ranging from 2516.587±17.983 (mg/100 g) in *B. longipes* to 583.317±9.505 (mg/100 g) in *V. amygdalina. Rhus natalensis* and *V. amygdalina* had low levels of Zinc. Anti-nutritive phytocompounds such as saponins and Tannins were also detected in some of the test extracts. The study concludes that plants used by pregnant women may have either a positive or negative effect on expectant mothers. There is need to formulate evidence based effective medicines for their safe use in the management of malaria.

Keywords: Medicinal plants, malaria, pregnant women, uterine contractility, micro-elements, anti-nutritive phytocompounds.

1. INTRODUCTION

Malaria is one of the most severe public health problems globally. It is present in 90 countries worldwide, with ninety percent of the cases being in Sub-Saharan Africa [2]. It is a major threat to child health while pregnant women are also particularly vulnerable to the disease [3]. In Uganda, the disease is the leading cause of morbidity and mortality with over 90% of the country being highly endemic. It is the leading cause of anemia among expectant mothers and the main reason for low birth weight in infants [1]. Maternal anemia and low birth weight as a result of prematurity and/or intrauterine growth restriction are the main adverse outcomes of placental infection and tend to be more severe in first pregnancies and in younger mothers.

The effective treatment of malaria in expectant mothers, especially in the rural areas of the country is limited by high malaria transmission intensity, inadequate health care resources, a weak health system, increasing resistance of the *Plasmodium* sp. parasites to the available allopathic anti-malarial drugs and the failure to eradicate the vector mosquitoes that transmit the disease [4]. Antimalarial drug resistance has spread and increased over the years leading to a dramatic decline in the efficacies of the available antimalarial allopathic medicines. The disease burden is aggravated by challenges in measuring mortality in Uganda due to lack of adequate data on determinants of death [5] let alone the propensity of death outside the formal health setting [6], some of which is composed of women who use medicinal plants with unproven scientific evidence during pregnancy. The use of plants in treatment of various diseases contributes significantly to primary health care in Uganda [7]. The extent of use of herbs in the treatment of malaria is particularly more in rural areas where there is a poor National Health Service delivery system hence lack of access to allopathic medicines. Poverty and the associated side effects of the available allopathic antimalarial drugs have further contributed to the increased utilization of medicinal plants by local communities that are thought to be free from side effects [8]. Several surveys have been carried out in Uganda to document the use of Herbal medicines in treatment of malaria [9,10,11,12, 13] and [14]. Several pharmacologically active anti-malarial compounds have been isolated from some plants and are at different stages of development [15,16,12,17] and [18]. However, not many studies have ascertained the health benefits including; potency, safety and efficacy of these plants in pregnant women.

Plants have been previously documented to be used regularly and intensively to achieve good reproductive health; including treatment of malaria in expectant mothers in Uganda and some have been proven efficacious against *Plasmodium falciparum* the parasite that causes the disease [19,14]. Furthermore, some of the plants are reportedly taken by pregnant mothers for healthy fetal development, blood tonics, and immune boosters and to accelerate the uterus settle in the post-partum period in order for the mother to normalize fast after child birth [20].

The plants under this study were *Bothliocline longipes, Vernonia amygdalina*, *Maesa lanceolata*, *Rhus natalensis* and *Trimeria bakeri*. Diethyl ether and methanol plant crude extracts were assayed against the uterine contractility in rabbit; Iron and Zinc were also quantified in order to determine if the plants are beneficial to pregnant women. Iron and zinc were chosen since they are key essential micronutrients in the prevention of anemia and immune boosting respectively.

2. METHODS

2.1 Study Design

This was an experimental laboratory-based study that investigated the effect of the leaf extract of the plants on the amplitude of contractility of an isolated rabbit uterus using the organ bath experimental setup. The plants under study were identified and authenticated by a taxonomist as: *Bothliocline longipes* Oliv. and Hiern, N. E. Br., (EKM015, Asteraceae family), *Vernonia amygdalina* Del. (EKM013, Asteraceae family), *Maesa lanceolata* Forssk (EKM045, Myrsinaceae family), *Rhus natalensis* Bernn. ex. Krauss, (EKM064, Anarcadiaceae family) and *Trimeria bakeri* Gilg. (EKM019, Flacourtiace family). Voucher specimen of all the plant species were deposited at the Makerere University Herbarium (MHU) for future reference. The laboratory work was done at Makerere University College of Health Sciences, Department of Pharmacology and Therapeutics, Kampala. While the zinc and iron analysis experiment was done at the Directorate of Government Analytical Laboratory in Wandegeya, Uganda to determine the presence and quantification of iron and zinc in the plants under study. Preliminary studies done on the different extracts of the study plants showed no observed uterine muscle contraction in the *B. longipes* crude extract. The extracts of the plant were therefore not analyzed further for the uterine contraction properties.

2.2 Sample Collection and Preparation

Fresh samples of *M. lanceolata*, *R. natalensis* and *V. amygdalina* were collected from Kagando village, Nyakayojo sub-county in Mbarara district. The plant materials (fresh leaves) were washed with distilled water to remove all the soil and impurities and they were then air dried in a dust free shade to constant weight. The dried materials were then grounded separately with a mortar and a pestle into a fine powder which was then placed in air tight labeled containers.

The powders of each plant were divided into two parts where one portion was used in the analysis of zinc and iron whereas the other was used in preparing the plant crude extracts which were then used in phytochemical screening and in the determination of their effects on the contractility of an isolated rabbit uterus *in vitro.*

The latter portion of the powdered materials was sequentially soaked in 500 ml of ether and methanol respectively. The mixtures were left to stand at room temperature for two days with occasional shaking. The extracts were then filtered into clean round bottomed flasks through Whatman Qualitative filter paper No. 1. The filtrate was concentrated using a rotary evaporator set at 40°C and 70°C for ether and methanol respectively. The final semi-solid extract obtained was put in an oven set at 40°C for 24 hours to obtain a stable solid extract. The weight of the crude extract was taken and the percentage (%) yield was calculated as = Mass of the extract obtained/Mass of original powder soaked x100.

2.3 Preparation of Stock Solutions of Different Plant Extracts

Ether and methanol leaf crude extracts (1 gm, each) of *V. amygdalina, M. lanceolata, R. natalensis* were put in falcon tubes. A few drops of dimethylsulfoxide was added in each to dissolve the extracts and topped up with 2 ml of distilled water to obtain a stoke solution of 500 mg/ml for each extract. Calibrated syringes were then used in obtaining the required concentrations of the extracts from the stock solutions.

2.4 Selection and Treatment of Experimental Animals

Two normal, healthy, non-pregnant, non-nursing adult Chinchilla Dutch rabbits weighing 3.1 kg and 3.4 kg were purchased from Nyenga, Jinja district and were housed in the animal unit of the Department of Pharmacology and Therapeutics, College of Biomedical Sciences, School of Health. The animals were treated according to international guidelines on laboratory animal use according to OECD [21] test No. 420. They were provided with food pellets from Engano Millers Limited (Nuvita), Kampala, Uganda and availed clean water for drinking. They were allowed to acclimatize for a period of two weeks before the experiment commenced [21].

2.5 Effects of Extracts on Rabbit Uterus

The rabbit's necks were completely dislocated, which caused massive injury to the brain stem and the spinal cord leading to immediate loss of consciousness. Uterine segments 3 cm in length were rapidly dissected out and were mounted on a hook inside a 20 ml organ bath apparatus using a small thread and a pin. The lower end of the tissue (uterus) was attached to the tissue holder by means of a thread and a pin while the upper end was also tied with a thread and connected to a small rod having a writing pen on the kymograph.

The bath containing the uterine segments was continuously supplied with a physiological salt solution maintained at 37^0C from the reservoir flask and air containing 95% oxygen and 5% carbon dioxide from a gas cylinder. The physiological solution was made by dissolving 40 g of sodium chloride, 1.0 g of calcium chloride, 5 g of sodium hydrogen carbonate, 1.0 g of potassium chloride, 0.25 g of sodium di-hydrogen phosphate, 0.5 g of magnesium chloride and 10 g of glucose in five liters of distilled water. The preparations were equilibrated for 45 minutes at resting tension before the start of the experiment. After equilibration, spontaneous contractions (amplitude and frequency) were recorded during the first 10 minutes with distilled water which enabled us to obtain the base-line for the different concentrations of oxytocin as a positive control and the test plant extracts. The maximum amplitude of contraction and the number of uterine contractions per four minute interval were used to calculate the average uterine contractions per given dose. Using SAS statistical package the mean and least significant difference values were used to compare different uterine contractility values of the different test extracts. It is however noted to our regret that in effort to obtain the study organs *ex vivo* in the nearest to their life state; the rapid neck dislocation in the study animals which weighed greater than 1 kg caused a lot of pain to the sacrificed study animals.

2.6 Qualitative Phytochemical Screening

2.6.1 Phytochemical test

The fleshly prepared diethyl ether and methanol plant crude extracts were subjected to standard qualitative phytochemical screening tests for the various constituents [22,23]. The extracts were screened for the presence of alkaloids, glycosides, saponins, tannins, anthraquinones and flavonoids among other compounds.

2.7 Iron and Zinc Analyses

The experiment was done using the atomic absorption spectrophotometer (AAS63000). To the second portion of the powdered plant material (2 g) of each plant part was added 25 ml of nitric acid and 5 ml of water, incubated for 4 hrs at 90°C, cooled and perchloric acid (10 ml) was added. The mixture was then heated at 90°C for 1 hr, cooled and hydrochloric acid (2 ml) was added and the contents were diluted up to 100 ml. The P-value less than 0.05 were considered statistically significant for mineral analysis.

3. RESULTS

3.1 Percentage Yield of the Plant Extracts

Results on the mass yield of the diethyl ether and methanol plant crude extracts are presented in this section.

Table 1. Percentage yield of the ether and methanol plant leaf crude extracts

Medicinal plant	Ether extract yield (%)	Methanol extract yield (%)
V. amygdalina	6.9	4.4
M. lanceolata	6.1	4.2
R. natalensis	6.3	5.3

Generally the ether extracts had a higher percentage yield compared to the methanol plant crude extracts. Amongst the ether plant crude extracts, the highest yield (6.9%) was observed in *V. amygdalina* followed by *R. Natalensis* (6.3%). The percentage yield range between the highest and lowest plant crude extracts was 0.8%. In the methanol plant crude extracts *R. natalensis* had the highest yield (5.3%) while the lowest was in *M. lanceolata* (4.2%). The percentage yield range calculated was 1.1%.

3.2 Effects of Different Doses of the Plant Crude Extracts and Oxytocin on Rabbit Uterus

Results on the effects of the different dose concentrations of the plant crude extracts and the average amplitude caused by oxytocin were observed as presented in Table 2.

Table 2. Effects of plant leaf crude extracts and oxytocin on the contractility of isolated rabbit uterus

Medicinal plant	Mean amplitudes of contraction of an isolated rabbit uterus at different concentrations (mm)		
Doses (mg/ml)	**0.2**	**0.4**	**0.8**
Methanol plant crude extracts			
V. amygdalina	18.2±0.80	25.5±0.70	37.8±1.26
M. lanceolata	ND	23±1.34	16.7±2.01
R. natalensis	ND	22±1.01	24.3±0.06
Ether plant crude extracts			
V. amygdalina	ND	26.0±0.98	22.5±1.10
M. lanceolata	ND	21.5±1.57	15.0±1.78
R. natalensis	ND	27.5±0.80	24.5 ±0.99
Oxytocin doses (I.U)			
I.U	17.5±0.02	22.8±0.57	31.0±0.36

Key: I.U –international units; ND-Not detected

The results showed that in all the plants tested, the ether and methanol plant crude extracts at 0.4 mm and 0.8 mm caused contractions of the isolated rabbit uterine muscle. At a concentration of 0.2 mg/ml the plant crude extract of *V. amygdalina* showed uterus contractility amplitude of 18.2 mm, no other plant crude extract had an effect on the uterus at this concentration apart from oxytocin which had 17.5 i.u. At a the concentration of 0.4 mg/ml, the methanol plant crude extract produced higher or close amplitude of rabbit uterine contraction in comparison to oxytocin. The methanol plant crude extracts of *V. amygdalina, M. lanceolata* and *R. natalensis* showed uterine contractility amplitudes of 25.5, 23.0 and 22.0 mm respectively compared to 22.8 i.u observed in oxytocin. *Vernonia amygdalina* and *R. natalensis* produced increased contractility amplitudes of 37.8 mm and 24.3 mm at 0.8 mg/ml, respectively while *M. lanceolata* showed a decline in the amplitude of contractility of 16.7 mm compared to an increase to 31.0 i.u observed in oxytocin.

The ether extracts of *V. amygdalina, M. lanceolata* and *R. natalensis* had no impact on the amplitude of contractility of the isolated rabbit uterus at the concentration of 0.2 mg/ml. With increased concentration to 0.4 mg/ml the plant crude extracts of *V. amygdalina, M. lanceolata* and *R. natalensis* produced contractility amplitudes of 26.0 mm, 21.5 mm and 27.5 mm respectively. Contraction of the uterine wall was stronger in *V. amygdalina* and *R. natalensis*. Oxytocin had contraction strength of up to 22.8 i.u. However, as the concentration was increased to 0.8 mg/ml, the ether plant crude extracts of *V. amygdalina, M. lanceolata* and *R. natalensis* produced lower amplitudes of uterine contractility of 22.5 mm, 15.0 mm and 24 mm respectively. An increasing trend in the amplitude of contractility of the uterus with an increase in the concentration of oxytocin was observed. The highest uterine contraction observed in oxytocin was 31 mm at 0.8 mg/ml while the least was 17.5 mm at 0.2 mg/ml.

Summary of results of the amplitude of uterine muscle contractility at different dosages is presented in Figs. 1 and 2.

There was no observed contraction of the rabbit uterus at 0.2 mg/ml. While the contractions were stronger at 0.4 compared to 0.8 mg/ml in all the diethyl ether plant crude extracts.

All the methanol plant extracts caused contraction of the rabbit uterine wall. The highest contractions were observed in *V. amygdalina*, followed by oxytocin. *Vernonia amygdalina* and oxytocin also showed a general increase in the contraction as the concentration was increased from 0.2 - 0.8 mg/ml.

There was a general increase in amplitude with an increase in oxytocin concentration as observed in Fig. 3. A similar trend was observed in the methanol crude extracts of *V. amygdalina* and *R. natalensis*. The observed amplitude of uterine contraction in *V. amygdalina* was higher than that of oxytocin in all the respective concentrations.

3.3 Results on the Phytochemical Screening of Ether Extracts of Different Plant Samples

Results of phytochemical compounds from methanol and ether plant crude extracts of *V. amygdalina*, *M. lanceolata* and *R. natalensis* are shown in Table 3.

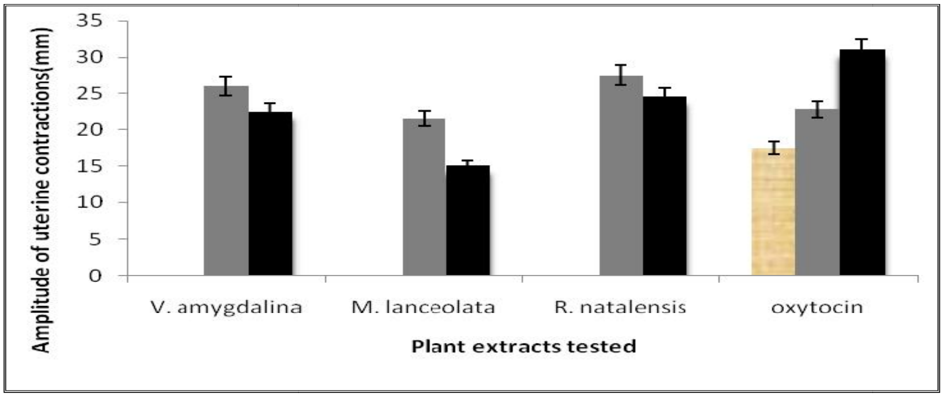

Fig. 1. Showing contractions of the diethyl-ether plant crude extracts and oxytocin at different concentrations

Key: Amplitude shown at different concentrations in mm; 0.2 0.4 0.6

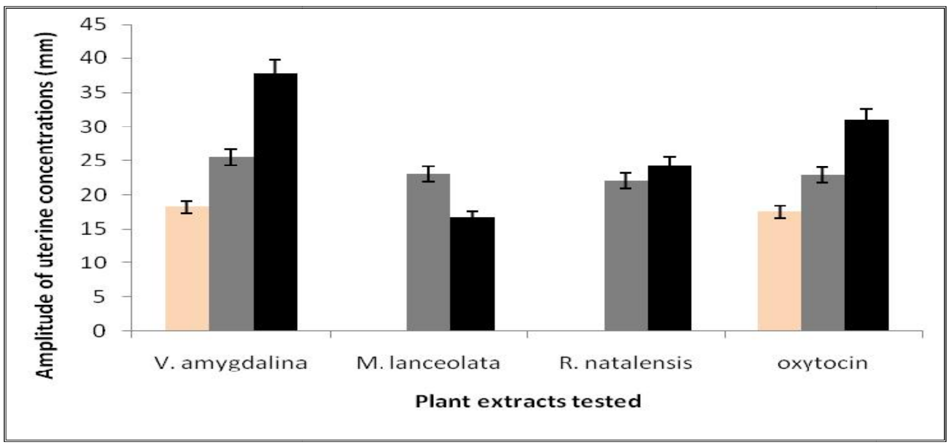

Fig. 2. Showing contractions of the methanol plant crude extracts and oxytocin at different concentrations

Key: Amplitude shown at different concentrations in mm; 0.2 0.4 0.6

In the ether plant crude extracts, *V. amygdalina* had saponins, flavonoids and alkaloids present while tannins, reducing sugars, anthraquinones and coumarins were absent. *Maesa lanceolata* had saponins, flavonoids, alkaloids, anthraquinones and coumarins present, while tannins and reducing sugars were absent. In *R. natalensis*, only flavonoids and alkaloids were present while tannins, saponins, reducing sugars, anthraquinones and coumarins were absent. The methanol extract of *V. amygdalina* contained tannins, saponins, flavonoids, alkaloids, reducing sugars and anthraquinones while coumarins were absent. Methanol extract of *M. lanceolata* had tannins, reducing sugars and anthraquinones present, alkaloids and coumarins were absent. While in the methanol extract of *R. natalensis* tannins, flavonoids and reducing sugars were present and saponins, alkaloids, anthraquinones and coumarins were absent.

3.4 Iron and Zinc Composition in Selected Plants

Results of iron and zinc concentrations from the named plants are shown in Table 4.

All the study plants contained large quantities of iron with *B. longipes* having the largest concentration followed by *M. lanceolata, R. natalensis* and *V. amygdalina* at 2516.587± 17.983 mg/100 g, 984.827±1.249 mg/100g, 806.862±4.606 mg/100 g and 583.317±9.505 mg/100 g respectively. Zinc was only detected in *R. natalensis* and *V. amygdalina* at 3.33±0.286 mg/100 g and 1.719± 0.286 mg/100 g respectively.

Table 3. Phytochemical screening of ether extracts of different plant samples

Plants	Phytochemical screened						
	Tannins	Saponins	Flavonoids	Alkaloids	Reducing sugars	Anthraquinones	Coumarins
Ether extract							
V. amygdalina	ND	+ + +	+	+ + +	ND	ND	ND
M. lanceolata	ND	+ +	+	+ +	ND	+ + +	+
R. natalensis	ND	+ +	ND	+ +	ND	ND	ND
Methanol extract							
V. amygdalina	+ + +	+ + +	+ + +	++ +	+ + +	+ + +	ND
M. lanceolata	+ +	+ +	ND	ND	+ + +	+ +	ND
R. natalensis	+ +	ND	+	ND	+ + +	ND	ND

Key: + + +; phytochemical present; ND - ; phytochemical not detected

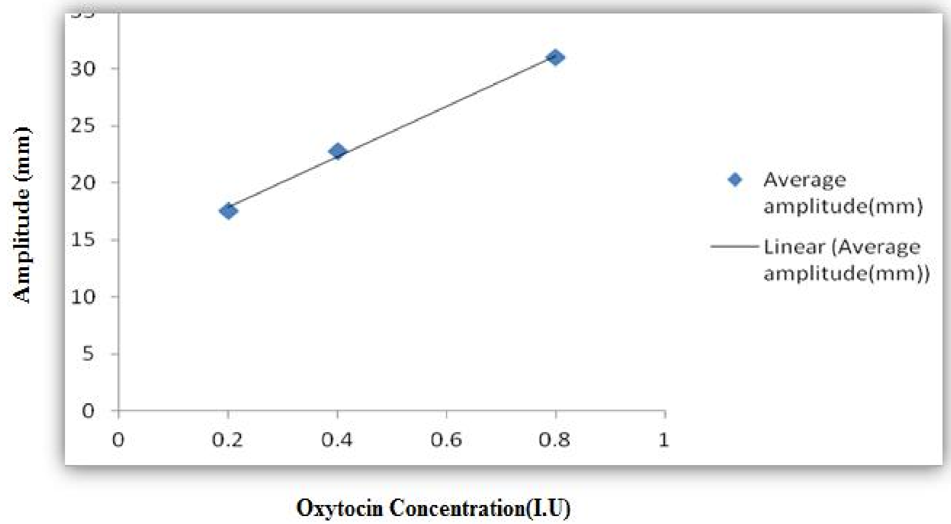

Fig. 3. A summary of the different oxytocin concentrations on the amplitude of contractility of the isolated rabbit uterus

Table 4. Total concentrations of iron and zinc the plants

Plant powder	Iron (mg/100 g)	Zinc (mg/ 100 g)
V. amygdalina	583.317 ± 9.51	1.719 ± 0.29
R. natalensis	806.862 ± 4.61	3.332 ±_0.17
M. lanceolata	984.827 ± 1.25	ND
B. longipes	2516.587±17.98	ND

Key: ND not detected by the machine

3.5 Analysis of Variance of Iron among the Selected Plants

A summary of the analysis of variance for statistical significance of iron in the study plants is shown in Table 5.

For results obtained in Tables 5 and 6, the mean difference was significant at 0.05 levels which meant that there was no correlation between iron and zinc concentrations among the selected medicinal plants which were analyzed.

4. DISCUSSION

Plants are used by majority of the people living in rural areas in treatment of various diseases in Uganda [24]. The use of plants in reproductive health including *V. amygdalina* has been documented [25,26]. Plants in this study including *B. longipes*, *V. amygdalina, M. lanceolata* and *R. natalensis* are used by pregnant women to manage malaria [14]. This study investigated the effect of ether and methanol crude extracts of the plants and oxytocin as a standard drug on the contractility of an isolated rabbit uterus. The results showed that methanol and ether leaf extracts of *V. amygdalina*, *M. lanceolata* and *R. natalensis* caused high amplitude of contractility in an isolated rabbit uterus similar to and in most cases higher than that observed in oxytocin. The results are in line with a previous study where the effect of *V. amygdalina* on uterine contractility *in vivo* was evaluated and found to be equally high [27]. The strong uterine contractions observed in these plants in this study could be the reason behind the assertions that herbal medicines are the major cause of miscarriages in expectant mothers, ruptured uteri at birth and child/mother high mortality rates, especially, in the rural parts of the country [28]. It is therefore important to document and standardize medicinal plants according to their correct use in order to benefit from their effective use in treatment of diseases particularly in the rural populations of Uganda where health infrastructure is still inadequate. Scientific validation of the traditional herbal medicines is a crucial step in improving management of diseases including malaria in

Table 5. Analysis of variance for statistical significance of iron in the selected plants

	Sum of squares	Df	Mean square	F	Sig.
Between Groups	6937870.667	3	2312623.556	1.565E4	.000
Within Groups	1181.925	8	147.741		
Total	6939052.592	11			

Table 6. Multiple Comparisons of iron in the plant samples

(I) Gp	(J) Gp	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
1	2	- 223.67500*	9.92440	.000	- 258.2008	-189.1492
	3	- 401.64333*	9.92440	.000	- 436.1691	- 367.1175
	4	-1933.40000*	9.92440	.000	-1967.9258	-1898.8742
2	1	223.67500*	9.92440	.000	189.1492	258.2008
	3	-177.96833*	9.92440	.000	- 212.4941	- 143.4425
	4	-1709.72500*	9.92440	.000	-1744.2508	-1675.1992
3	1	401.64333*	9.92440	.000	367.1175	436.1691
	2	177.96833*	9.92440	.000	143.4425	212.4941
	4	-1531.75667*	9.92440	.000	- 1566.2825	- 497.2309
4	1	1933.40000*	9.92440	.000	1898.8742	1967.9258
	2	1709.72500*	9.92440	.000	1675.1992	1744.2508
	3	1531.75667*	9.92440	.000	1497.2309	1566.2825

*. The mean difference is significant at the 0.05 level.

Key: Gp - group; 1- V. amygdalina; 2 - R. natalensis; 3 - M. lanceolata; 4 - B. longipes pregnant women in order to prevent miscarriages, child-birth related complications and some mother and fetal health defects.

The reason for the high amplitude of contractility in the methanol leaf extracts of *V. amygdalina and R. natalensis* could be attributed to the many phytochemicals present in methanol extracts which could have interacted with the uterine walls to bring about the increase as opposed to those in ether plant extracts. The methanol extract (0.4 mg/ml) of *M. lanceolata* maintained high amplitude of contractility for a longer time before returning to the base-line. Maintenance of a high amplitude of contractility by the extract for a relatively long time could be attributed to some special unique phytochemical with this property which is only available in *M. lanceolata.* The methanol leaf extracts of *V. amygdalina and R. natalensis* caused a greater increase in the amplitude of contractility of the isolated rabbit uterus in a dose-dependent manner and higher than that caused by oxytocin. Diethyl ether crude extract of *M. lanceolata,* on the other hand, showed a decrease in the amplitude of contractility of the isolated rabbit uterus. Uterotonic agents, including diterpenes, phenyl-propanoid glucosides, heterocyclic aldehydes, fatty acids, saponins, sterols and polypeptides, were found responsible for the contractile properties of the plant species in a study in Nigeria [27]. In this study, saponins could be responsible for the contractile effect that was observed in the validated effect of the rabbit uterine muscle. There is need to analyze the contractility effect of the other compounds that were found present to ascertain their individual effects on the contraction of the rabbit uterine wall; this is because contraction was also observed in *R. natalensis* that did not have saponins. Quantification of the compounds that are found to cause uterine contractions is also important. The obtained knowledge will form a basis for the formulation and standardization of products for effective and safe use by the pregnant women. Chemicals and drugs, including those of plant origin are known to cause negative birth and fetal development effects [29]. It is therefore important that teratogenic safety studies are carried out for safe use of these plants during pregnancy. It is possible that the ether extracts contain compounds which cause uterine membrane polarization and the closure of the voltage-gated L. type calcium channels which leads to low calcium entry. Hence, this may explain a decline in the amplitude of contractility [30]. The observed overall increase in the amplitude of contractility of the isolated rabbit uterus using the methanol leaf extracts of *V. amygdalina, R. natalensis* may have, however, been due to the agonistic effect of the compounds in the leaf extracts.

Plant leaf extracts have also been reported to contain carbohydrates, ascorbic acids, protein, fiber, vitamins, minerals and micronutrients in varying quantities [31,32]. Minerals such as calcium play a role in uterine contractions. The increase in the permeability of the calcium ions may be responsible for the increase in the amplitude of contraction of the uterus because the increased concentrations of calcium ions plays a major role in the contractile process of myofibrils [33,34,35]. In this study, a high concentration of calcium ions from the extracts that were introduced around the uterus increased the Ca ions outside the cell membrane and could have lead to more calcium entrance following a concentration gradient hence increasing the amplitude of contractility of the uterus. Various minerals including calcium (Ca^{2+}), potassium (K^{+}), sodium (Na^{+}), and magnesium (Mg^{2+}) in various quantities with potassium and calcium ions being higher than sodium, iron and magnesium have been reported in *V. amygdalina* [31,32]. On the other hand, presence of micronutrients such as iron and zinc could be effective for blood generation and immune boosting properties respectively. In this study, the leaf extracts of *V. amygdalina, M. lanceolata, R. natalensis* and *B. longipes* were found to have large quantities of iron; the highest being observed in *B. longipes.* Small quantities of zinc were also observed at 1.719 ± 0.286 mg /100 g and 3.332 ± 0.167 mg /100 g in *V. amygdalina and R. natalensis* respectively. The importance of these plants in prevention of anemia and well being of a pregnant woman can therefore not be over emphasized. This is particularly so for the rural women who may not have access to quality health care facilities and iron supply, more especially, in the first trimester since they report late to the available health centers due to the long distance sometimes. Taking high dosages of these plants in the early stages of pregnancy could result in miscarriages. Moderated quantities of these plants in the late trimester, however, could lead to enhanced labor, increased blood and immunity in expectant mothers. On the other hand, some of the plants were found to have potentially dangerous phytochemicals such as saponis, tannins etc. Saponins have been reported to hymolyse human erythrocytes *in vitro,* while tannins have tannic acid that negatively affects blood proteins [36,37]. Quantification of such phytocompounds in the leaves of these plants should be done in order to determine their safe use in humans particularly the pregnant mothers. Sometimes the preparation of these herbs by the local people is helpful since in some cases the toxicity can be removed /reduced during preparation of the herbal therapies. The study highlights the need for continued research on the herbal extracts such that commendable antimalarial/nutritional herbal products for expectant mothers in local communities are formulated.

5. CONCLUSION

Results from this study serve as a starting point in the search for safe, longer lasting, effective, immune boosting, blood tonic and tolerable uterotonic medicine leads and products in plants. The information obtained is required for the standardization and use of herbs in evidence based treatment of malaria, particularly, the pregnant women who are majorly affected by the disease in rural communities of Uganda.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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