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Modulation of uterine contractility in the isolated mouse uterus by the methanol extract of Talinum Triangulare (Portulaceae) and investigation of significant secondary metabolites

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ABSTRACT

Talinum triangulare is used by some traditional healers to manage preterm labour and prevent preterm birth. The effect of *T. triangulare* on uterine contractility is so far unknown. This study was therefore aimed at: the investigation of the pharmacological and physiological effect of *T. triangulare* leaf extract (MTT) on the isolated mouse uterus; investigation of possible mechanisms of activity and identification of significant metabolites present in the leaves. Longitudinal uterine segments from non-pregnant mice were isolated and mounted in a continuously aerated organ bath containing physiological salt solution and maintained at 37°C. The effect of MTT (0.0007 -7.777 µg/ml) on spontaneous contractions, 11.82 nM oxytocin, and 40 mM KCl was investigated. Additionally, MTT (3.43 mg/ml) in the presence of 95.34 nM amiodarone, 5.06 nM glibenclamide and 11.57 nM propranolol was investigated. High resolution mass spectrometric (HRMS) analysis was performed on MTT. MTT exerted a biphasic effect on spontaneous uterine contractility, with low concentrations inhibiting and high concentrations stimulating contraction. MTT also increased oxytocin and KCl-induced contraction. The effect on oxytocin was reduced in the presence of amiodarone but not glibenclamide or propranolol. HRMS identified high levels of oestrogenic compounds which may play a role in the activity of the plant on female reproductive health.

KEYWORDS: *Talinum triangulare*; uterus; oxytocin

INTRODUCTION

One of the problems faced by obstetricians today is preterm birth which is birth occurring at less than 37 weeks gestation [1]. A large number of infants die annually due to preterm birth complications [2] which makes the need for better treatment options and medicinal plants are viable sources for the search of potential remedies. One plant used traditionally in the prevention of miscarriage in Nigeria, is the plant *Talinum triangulare*. *T. triangulare*. Leach is commonly known as "water leaf"' vegetable in Nigeria. It is a commonly used vegetable in soups, stews and salads in Nigerian cuisine. It has been described as an edible plant which falls under the cosmopolitan herbaceous group of plants and flowers that grow perennially. The roots of the plant are enlarged and it possesses a succulent stem which can grow up to 30-100 cm tall [3]. *T. triangulare* is of the family Portulaceae and is well distributed in tropical parts of Africa such as Nigeria, Cameroon, Ghana, and in other regions like the south East Asia and the Amazon River margins [4,5].

Interestingly, this common vegetable has been reported to have medicinal effects. For instance, the aqueous root extract has been reported to have anti-diarrheal effects [6], antioxidant and hepatoprotective effects [7], as well as antidiabetic effects [8]. Extracts of the stem have been reported to show immunomodulatory effects [9] and the aqueous leaf extract have also been reported to exhibit anti-ulcer effects [10]. Traditional healers in Southern Nigeria report the use of the plant to prevent 'miscarriage' (personal communication with traditional healer, Mr. Eyohan of Edo State Nigeria). The plant is prepared for use in pregnant women by

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first washing and then soaking the leaves of the plant in water for a while. A small glassful (approximately 250 ml) of the decoction is taken three times a day or as needed. The leaves can be used either fresh or dry. The plant was also reported to be used by women with a history of miscarriage, where a small glassful is taken in the morning before meals (personal communication). Detailed search of the literature showed that no previous study had been done to investigate the effect of the plant on uterine contractility.

Previous studies had however reported the presence of several secondary metabolites isolated from different parts of the plant. The secondary metabolite classes so far reported based on a preliminary phytochemical studies include alkaloids, flavonoids, glycosides, reducing sugars, saponins, proteins, carbohydrate, fats and oils, tannins, steroids, resins and terpenoids [11]. Isolation of an acrylamide and some phaeophytins have also been reported [8].

This current study is therefore aimed at evaluating the potential benefit of the plant in controlling uterine contractility which may be essential in the management of conditions such as preterm labour or dysmenorrhoea where uterine contractility is affected. This study utilizes an *ex-vivo* model in order to investigate the effect of an extract of the plant on uterine contractility. This study is also aimed at investigating possible mechanism(s) of activity associated with the effect observed as well as providing knowledge for further research.

MATERIALS AND METHODS

Plant material

Fresh leaves of *T. triangulare* were collected in the month of March from Egor Local Government Area in Benin City, Edo state, Nigeria. The plant was identified by Dr. H. A. Akinnibosun from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, and by Professor B.A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy University of Benin, Benin City, Nigeria.

Plant extraction

The leaves of the plant were carefully separated from the stem, while debris and contaminants were also removed. The fresh leaves were chopped into small pieces and macerated in absolute methanol of analytical grade. Methanol was chosen as a solvent for extraction due to the similarity in polarity to water [12] and because it is easier to evaporate under laboratory conditions compared to water. Maceration was performed for 48 h at room temperature of approximately 29 ± 3°C with continuous stirring. After 48 h, the mixture was decanted and filtered. The filtrate was concentrated using a rotary evaporator set at 60°C and subsequently dried in an oven set at 40°C. The resulting solid weighed 2.192 g resulting in a percentage yield of 3.1%. The dried extract was placed in an air-tight container and stored at 4°C till needed. It is important to note that the solvent was completely evaporated off, leaving the solid extract for further studies.

Animals

Non-pregnant female Swiss albino mice weighing between 24.0 - 30.0 g were obtained from the Animal House Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Edo state, Nigeria. They were housed in plastic cages at an environmentally controlled room temperature of approximately 28 °C ± 5 °C. The animals were handled as much as possible according to standards set by the Public Health Service policy on humane care and use of Laboratory Animals 2002. Ethical clearance was obtained from the animal use ethical Committee of the Faculty of Pharmacy, University of Benin, Nigeria, and reference number EC/FP/016/06. The animals were maintained on standard diet of animal pellets and clean tap water.

Contractility Studies

Tissue preparation

Twenty four hours prior to the day of experiments, each mouse was orally administered 1.0 mg/kg diethylstilbestrol (DES) constituted in 5% Tween 80 and distilled water (1:1) using a feeding syringe. This dose and route of administration had been previously determined in our laboratory to effectively induce oestrous phase of the oestrous cycle. The use of DES in this study was to initiate conditions of labour that would otherwise have occurred in the pregnant mice if conditions of preterm labour or labour itself were initiated. It is well known that there is a shift in circulating hormones from progesterone to oestrogen prior to labour [13,14]. On the day of the experiment vaginal smears were obtained by flushing with distilled water using a Pasteur pipette (0.1 mm). A smear was made on a clean glass slide, fixed with ethanol and stained with a drop of Gentian violet. The smear was then viewed under a microscope using a x10 objective lens. As soon as the stage of

oestrous had been ascertained, the mouse was then humanely killed by cervical dislocation and the uterine horns were immediately excised and placed in a petri-dish containing previously warmed and aerated physiological salt solution (PSS) of the following composition in g/5 L: NaCl 45.0, $NaHCO_3$ 2.5, D-glucose 2.5, KCl 2.1, and $CaCl_2 \cdot 2H_2O$ 1.32. The uterine tissues were cleaned of connective tissues and one horn was cut medially in half and lengths of approximately 1-2 mm each were obtained. The selected uterine segment was then mounted in a warmed 10 ml organ bath maintained at 37°C and containing aerated physiological salt solution of the composition earlier described. The mounted tissues were equilibrated under resting tension of 0.5 g for 30 min. The force and frequency of uterine contractions in the longitudinal muscle layers were measured using a 7003E-isometric force transducer (UgoBasile, Varise, Italy) connected to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (UgoBasile, Varese, Italy).

Drugs and chemicals

All chemicals used were of analytical grade and were purchased from Pharmatrends (Benin City, Nigeria). Necessary stock solutions were prepared and stored in adherence to the manufacturer's guidelines. Other drugs were purchased as described, Oxytocin (OT) (Pantocyn®, Jiangsu Ruinian Qianjin Pharmaceutical Co. Ltd. China), amiodarone (TEVA UK. Ltd), glibenclamide (Daonil®, Swiss Pharmaceutical Nigeria, Ltd.), and propranolol (Sigma-Aldrich, UK).

Experimental protocols

All protocols were performed after equilibration and after regular contractions were achieved as much as possible. Preliminary investigations were first performed to determine concentrations and to determine appropriate or suitable duration for each drug concentration. Time-matched controls where used to also confirm the allowable duration of the control drugs oxytocin and KCl (Data not shown). Time periods therefore reported had been found suitable for determining drug effect under cumulative concentration-response evaluation within the conditions of this study.

MTT on Spontaneous Uterine Contraction

Cumulative concentrations of MTT (0.0007-7.777 mg/ml) were added to the isolated uterine tissue and concentration-response relationships were obtained. A contact time of 3 min was allowed following each concentration of extract administered. After each set of administration, the tissues were washed 3 times and tissue recovery was monitored. Regular spontaneous contractions occurring 3 min before extract addition, was taken as 100% control.

MTT on Oxytocin (OT)-induced Uterine Contraction

On attainment of steady spontaneous contractions, the uterine tissues were stimulated with OT (11.82 nM) for 5 min to obtain regular phasic contractions. MTT (0.0007-7.77 mg/ml) was then cumulative added in the continued presence of oxytocin for 3 min per concentration. Immediately after the experiments, the uterine tissue was washed with the PSS and allowed to rest and recover. The last 3 min of OT prior to addition of the extract was taken as 100% control.

MTT on High KCl-induced Uterine Contractility

The effect of the extract was determined in the presence of concentrated KCl (40 mM). This was done in order to determine the effect of MTT on the depolarized uterine tissue. KCl was added to the bath containing the uterine tissues for 5 min and in the continued presence of KCl, cumulative concentrations of the extract (0.034 – 343.33 mg/ml) were added for 3 min per concentration. At the end of the experiment, the tissue was washed with PSS and allowed to recover. The last 3 min of KCl prior to addition of MTT was taken as 100% control.

MTT on OT-induced contractions in the absence and presence of receptor blockers

In separate experiments, the effect of a submaximal concentration of the extract (3.43 mg/ml) on OT-induced contraction (11.82 nM) was determined in the absence and presence of amiodarone (95.34 nM), glibenclamide (5.06 nM) and propranolol (11.57 nM). After steady spontaneous contractions were obtained, OT was added to the tissue bath for 5 min and in the continued presence of oxytocin, MTT was added and left for a further 5 min. In the continued presence of OT and MTT, amiodarone, glibenclamide or propranolol was added to the tissues and observed for a further 5 min.

LC-HRFTMS identification of constituents in extract

Liquid chromatography-high resolution Fourier Transform mass spectrometry (LC-HRFTMS) analysis was performed using a Dionex UltiMate-3000 (DIONEX, Sunnyvale, CA, USA) coupled to a ThermoScientific Exactive Orbitrap system (Thermo

Fisher Scientific (Bremen) GmbH, Bremen, Germany). The column used was a C18 75 × 3.0 mm column (Hichrom Ltd., Reading, UK). Compounds were eluted at a flow rate of 300 µL/min using water (A) and acetonitrile (B), both of which contained 0.1% formic acid. The gradient started with 10% B and increasing to 100% B in 30 min. The mobile phase was maintained at 100% B for 5 min after which the column was equilibrated with 10% B. The files were sliced into positive and negative data sets using ProteoWizard [15] prior to data mining using MZmine 2.10 [16]. Peak detection was accomplished using the centroid mass detector and a noise level of 1000. The chromatogram builder generated peak lists from the mass lists obtained from the previous step. The minimum time span was 0.2 minutes, minimum height was 10,000, and the *m/z* tolerance was set to 0.0001 *m/z* or 5 ppm. Chromatogram deconvolution was accomplished using the local minimum search algorithm with the following parameters: threshold (90%), search minimum in RT range (0.4 min), minimum relative height (5%), minimum absolute height (10,000), minimum ratio of peak top/edge (2), and peak duration range (0.2-5.0 min). The peak lists were de-isotoped using the isotopic peaks grouper with an *m/z* tolerance of 0.001 *m/z* or 5 ppm, retention time tolerance of 0.1 minutes (absolute), and maximum charge of 2. The representative isotope was the most intense. The peak lists were then merged using the Alignment function. The weight for *m/z* and for RT was 20, and the RT tolerance was 5%. The aligned peak lists were gap-filled using the Peak Finder, with an intensity tolerance of 1% and RT tolerance of 0.5 min (absolute). Adducts were identified, together with other complexes that may have formed. The chemical formulas of each peak were predicted using the formula prediction tool developed by MZmine. Hits from the database [17] were accessed using ChemBioFinder version 13 (PerkinElmer Informatics, Cambridge, UK).

DATA ANALYSIS

The mean frequency, and amplitude were calculated from contractions occurring at the last 3 minutes of the phasic contractions using the GraphPad Prism, (version 7.0; GraphPad software Inc, San Diego, CA, USA). Results were obtained as percentages of control applications (control=100%). In some experiments, changes in force or amplitude were expressed with respect to basal (resting) force level or basal agonist level (100%). All data shown were expressed as mean ± standard error (SEM) and 'n' represents the number of samples each from different animals. Significance was evaluated using appropriate t-tests and where necessary, one way analysis of variance (one-way ANOVA) with Dunnett's post hoc and P values ≤ 0.05 was taken to represent minimum significance in all cases.

In datasets with sufficient data points, mean log concentration-response curves were analyzed by fitting data to a four-parameter logistic equation, using non-linear regression with GraphPad Prism 7.0 (GraphPad software, San Diego, CA, USA) using either of the following equation values (Y = Bottom)/(1+10^((LogEC50-X)*HillSlope)). Where Y = response which starts at the bottom and goes to the Top in sigmoid shape, X= logarithm of concentration and IC_{50} is the concentration that produces half the maximal responses; and Span1=Plateau1-Dip; Span2=Plateau2-Dip; Section 1 = Span 1 (1+10^ ((Log EC_{50}_1-X) * nH1)); Section 2 = Span2/(1+10^ ((X-LogEC_{50}_2)*nH2)); Y = Dip + Section 1 + Section 2. Where Plateau 1 and Plateau 2 are the plateaus at the left and right ends of the curve, in the same units as Y and Dip is the plateau level in the middle of the curve, in same units as Y. LogEC_{50}_1 and LogEC_{50}_2 represent concentrations that give half-maximal stimulatory and inhibitory effects in the same units as X while nH1 and nH2 represent the slope factors.

RESULTS

Where necessary, an important observation was stated though statistical significant differences were not observed. This was necessary in order to emphasize on the importance of biologic significance in the absence of statistical significance [18–20].

Effect of MTT on Spontaneous Uterine Contractions

MTT at concentrations used in this study, was seen to cause a biphasic effect on spontaneous uterine contractions (Fig. 1A). At lower concentrations of 0.00071, 0.0071 and 0.014 mg/ml MTT, significant inhibition of the amplitude of contractions were observed (P = 0.0453 (P < 0.05); P = 0.0055 (P < 0.01); P = 0.0042 (P < 0.01) respectively) (Fig. 1B). However, higher concentrations of 0.079 -7.77 mg/ml attenuated the inhibition (Fig. 1B). A similar biphasic response was observed with the frequency of contractions. Lower concentrations of MTT (0.00071 – 0.014 mg/ml) inhibited frequency of spontaneous contractions while higher

concentrations overcame the inhibition produced by the lower concentrations (Fig. 1C). The EC_{50} of MTT was determined for the amplitude of contraction to be 0.00021 ± 0.66 mg/ml for the inhibitory phase and 0.063 ± 0.45 mg/ml for the stimulatory phase. The EC_{50} of MTT was also determined for the frequency of contraction and found to be 0.00047 ± 1.04 mg/ml for the inhibitory phase and 0.26 ± 0.60 mg/ml for the stimulatory phase. It is important to note at this point that though no statistical significant difference was observed at the attenuation of the inhibition produced by the lower concentration by higher MTT concentrations, we indicated nonetheless that there was an increase in contraction at those higher concentrations.

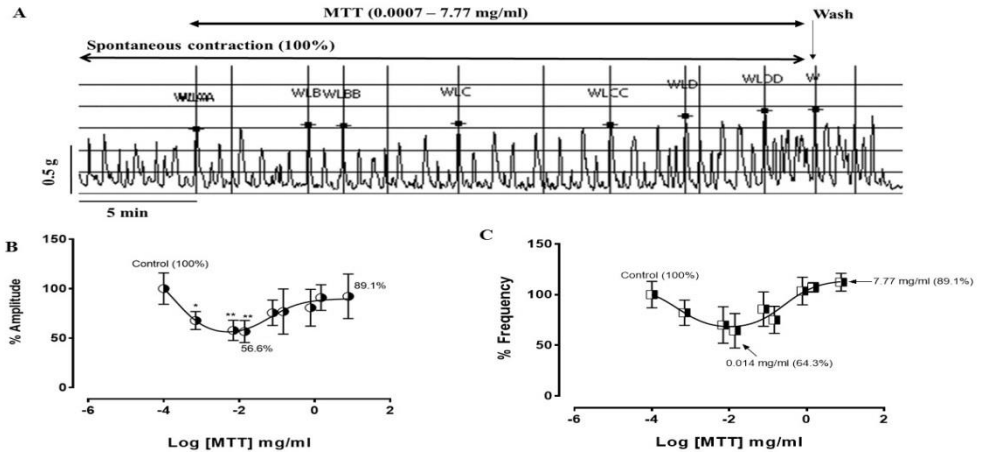

Figure 1. The response of spontaneous uterine contractility to MTT. The top panel shows an original recording of MTT on spontaneous uterine contraction (A). Concentration-response curves showing the effect of the extract on the amplitude of spontaneous uterine contractions (B) and the frequency of contractions (C) are shown. n = 5 animals *p< 0.05; **p < 0.01 compared to control.

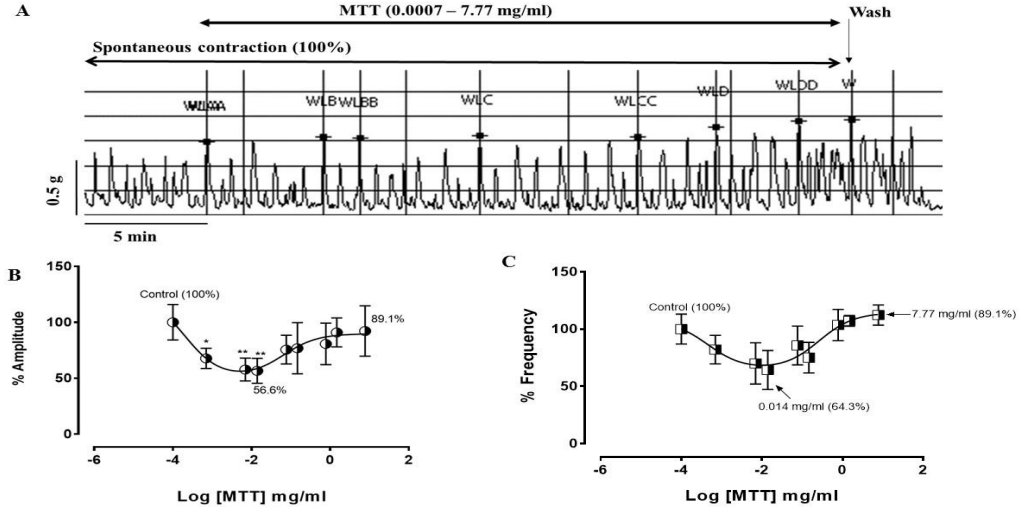

Figure 2. The response of OT-induced uterine contractility to MTT. The top panel shows an original recording of MTT on OT-induced uterine contraction (A). Bar plots showing the amplitude response of OT in the presence of MTT are displayed in panel B. Bar plots showing the frequency response of OT in the presence of MTT are displayed in panel C. An increase in amplitude of up to 48% was observed while an opposing decrease in frequency was observed. n = 5 animals; ns = not significant.

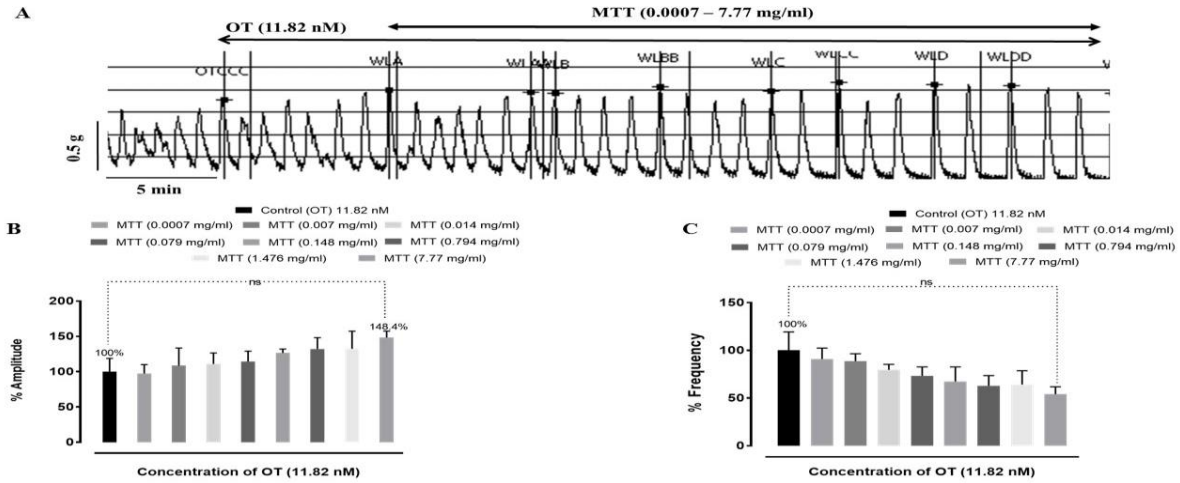

Figure 3. The response of KCl (40 mM) to different concentrations of MTT. Original recording showing the effect of MTT (0.34 – 343.33 µg/ml) on KCl-induced uterine contractions is shown in panel A. Bar plots showing a gradual increase in the amplitude of KCl to MTT is displayed in panel B. n = 5 animals.

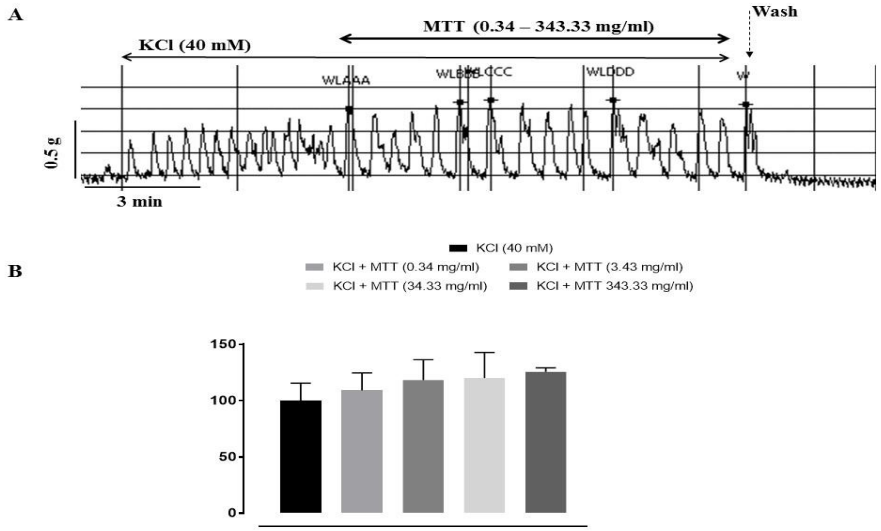

Figure 4. The response of OT-induced contractions in the presence of MTT and amiodarone (95.34 nM). Original recordings of the effect of OT in the absence and presence of MTT (3.43 mg/ml) and then amiodarone is shown in panel A. Bar plots showing the amplitude (A) and frequency (B) of OT-induced response in the presence of MTT and then amiodarone are shown. The effect of MTT was attenuated in the presence of amiodarone. n = 5 animals.

Table 1: Putatively Identified Compounds in MTT

	Compound Name	Molecular formula	Molecular weight (g/mol)	m/z	Rt (min)
1	2-Acetyl-6-ethyl-3-hydroxypyridine	$C_9H_{11}NO_2$	165.0792	$[M+1]^+$166.0865	2.53
2	Citric acid	$C_6H_8O_7$	192.0273	$[M-1]^-$ 191.0200	1.24
3	1-O-Caffeoylquinic acid	$C_{16}H_{18}O_8$	338.0999	$[M-1]^-$ 337.0926	4.84
4	9,13-Dihydroxy-10-oxo-11-octadecenoic acid	$C_{19}H_{34}O_5$	342.2399	$[M-1]^-$ 341.2326	13.45
5	17-Hydroxyingenol	$C_{34}H_{40}O_9$	592.2689	$[M+1]^+$593.2763	29.33
6	Phaeophorbide a	$C_{36}H_{38}N_4O_5$	606.2848	$[M-1]^+$ 607.2621	32.14
7	Phosphatidylcholine	$C_{44}H_{76}NO_8P$	777.5301	$[M+1]^+$778.5374	26.62
8	Glycerol 1,3-didocosanoate 2-ferulate	$C_{57}H_{100}O_8$	912.7403	$[M+1]^+$913.7476	36.84

m/z = mass to charge ratio; Rt = retention time

Table 2: Unidentified Compounds Detected in MTT

	Predicted formulae	Molecular weight (g/mol)	m/z	Rt (min)
1	$C_9H_6N_6O_2$	230.0556	$[M-1]^-$ 229.0483	1.18
2	$C_{18}H_{32}O_5$	328.2244	$[M-1]^-$ 327.2171	11.09
3	$C_{27}H_{30}O_{15}$	593.1511	$[M+1]^+$594.1584	6.09
4	$C_{56}H_{92}N_6O_5$ $C_{51}H_{92}N_8O_7$	928.7093	$[M+1]^+$929.7165	37.33

m/z = mass to charge ratio; - = unknown; Rt = retemtion time

Effect of MTT on Oxytocin-induced Uterine Contractions

The effect of MTT in the presence of an agonist, oxytocin (OT) was investigated. MTT appeared to increase the force but decrease the frequency of OT-induced uterine contractions (Fig. 2A). A plot of the amplitude response to the concentration of MTT showed a gradual increase in the force of contraction (Fig. 2B) while a plot of the frequency response to the concentration of MTT showed a gradual decrease (Fig. 2C).

Effect of MTT on KCl-induced Uterine Contractions

MTT displayed a gradual increase in the contractility induced by KCl (40 mM) (Fig. 3) which was attenuated when the tissue was washed (Fig. 3).

Effect of MTT on Oxytocin-induced Contraction in the Presence of Amiodarone, Glibenclamide and Propranolol

The stimulatory effect of MTT (3.43 mg/ml) on the amplitude of OT-induced contractions were slightly inhibited in the presence of amiodarone (Fig. 4). A decrease in both the amplitude (Fig. 4B) and frequency (Fig. 4C) were observed in the presence of amiodarone (95.34 nM).

However, glibenclamide (GBL) and propranolol (PPL) did not appear to have an effect on the MTT augmented increase of OT-induced contractions (Fig. 5A and 6A). This was also evident in the concentration-response column plots of both the amplitude (Fig. 5B and 6B) and the frequency (Fig. 5C and 6C).

Identified secondary metabolites in MTT

The LC-HRFTMS results and database search (using Dictionary of Natural Products) enabled the detection of twelve (12) significant compounds (Tables 1 and 2), four of which were unidentified (Table 2). The identified compounds include, 2-Acetyl-6-ethyl-3-hydroxypyridine (1), citric acid (2), 1-O-Caffeoylquinic acid (3), 9,13-Dihydroxy-10-oxo-11-octadecenoic acid (4), 17-Hydroxyingenol (5), phaeophorbide a (6), phosphatidylcholine (7) and glycerol 1,3-didocosanoate 2-ferulate (8). (Fig. 7)

DISCUSSION

The extract of *T. triangulare* (MTT) was observed in this study to exhibit a biphasic effect on spontaneous uterine contractions. To the best of our knowledge this is the first report on *T. triangulare* on uterine contractility. The biphasic effect observed showed inhibition at lower concentrations and as the concentration increased, the inhibition was attenuated and stimulation of contractility occurred as seen in this study. While no concrete investigations were done to adduce reasons for the biphasic effect observed, certain hypotheses can be drawn from the outcome of this study which can provide a basis for further investigation.

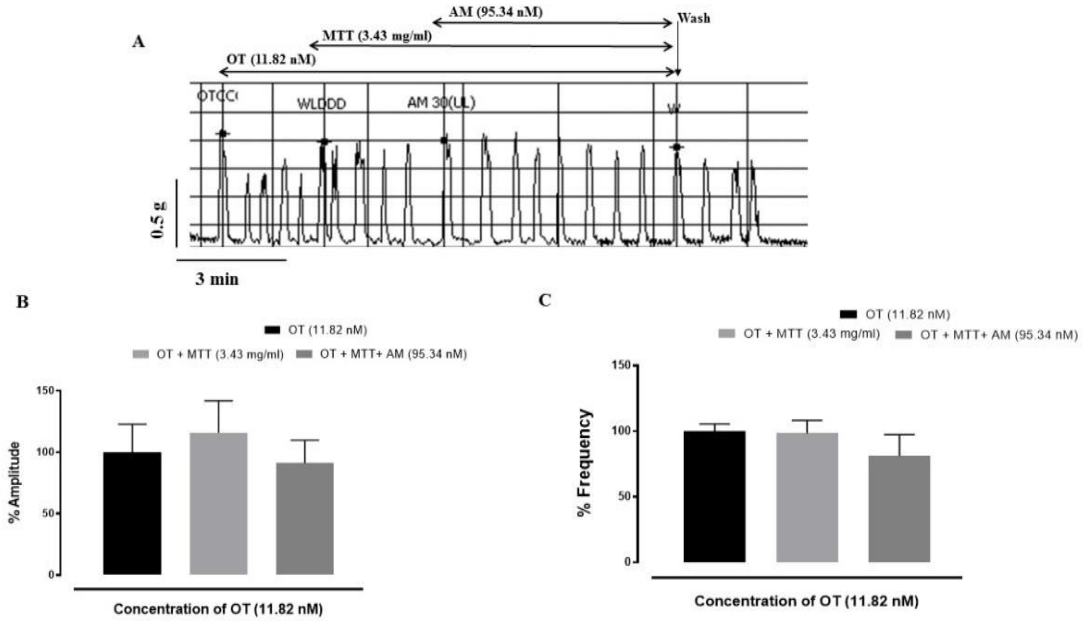

Figure 5. The response of OT-induced contractions in the presence of MTT and glibenclamide (5.06 nM). Original recordings of the effect of OT in the absence and presence of MTT (3.43 mg/ml) and then glibenclamide is shown in panel A. Bar plots showing the amplitude (A) and frequency (B) of OT-induced response in the presence of MTT and then glibenclamide are shown. In the presence of glibenclamide, the OT-response to MTT was unaffected. n = 5 animals.

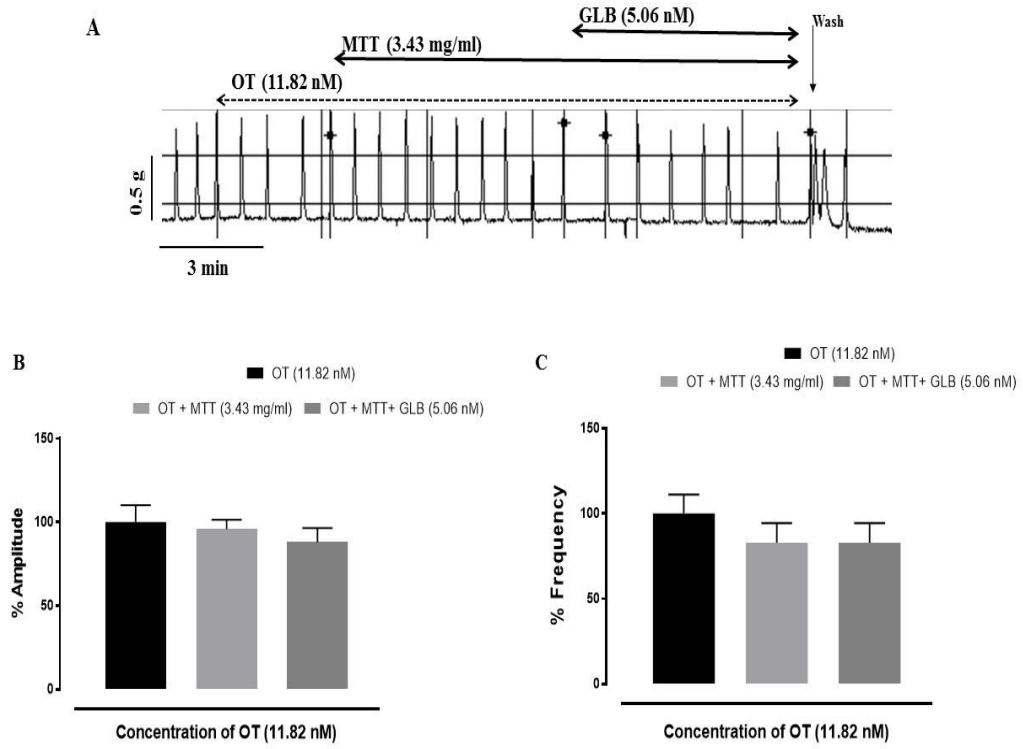

Figure 6. The response of OT-induced contractions in the presence of MTT and propranolol (11.57 nM). Original recordings of the effect of OT in the absence and presence of MTT (3.43 mg/ml) and then amiodarone is shown in panel A. Bar plots showing the amplitude (A) and frequency (B) of OT-induced response in the presence of MTT and then propranolol (PPL) are shown. In the presence of propranolol, the OT-response to MTT was unaffected. n = 5 animals.

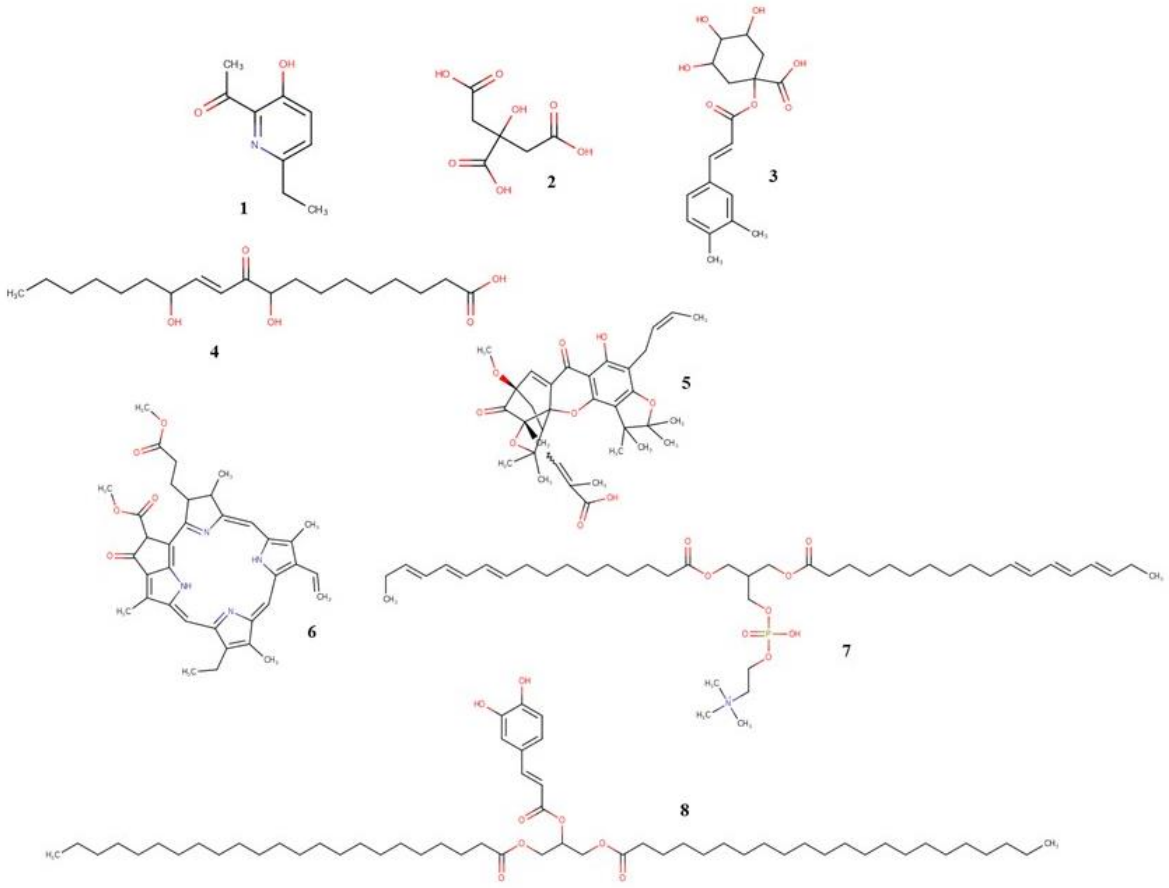

Figure 7. Chemical structures of putatively identified compounds detected after LC-HRFTMS analysis.

One possible cause attributable to this biphasic effect is the existence of different receptors or substrates to MTT which generates opposing cell-signaling pathways, or which generates a condition of activity-dependence [21] in response to MTT. Some studies where in vitro biphasic activity was investigated in detail, reported the existence of separate receptor populations within the same tissue but with different sensitivity to the drug being investigated [21]. This observation can therefore lead to the differential participation of the inhibitory and stimulatory G-proteins where G_i is activated at low concentrations of MTT and G_s is activated at higher concentrations of MTT [22]. This biphasic effect may also result from distinct anatomical presence of same receptors within the same tissue which where this occurs would lead to differential responses based on the G-protein network existing in the particular anatomical area and also depending on the magnitude of concentration required for their activation in the separate areas [21].

Therefore under specific conditions one would expect adenylate cyclase activation at some point and at another a phospholipase C activation. Biphasic drugs are beginning to gain considerable attention in clinical pharmacology and have been shown to play significant roles in certain pathologies including pain [23].

With regards to effect on agonist stimulation, MTT was observed to increase the force and frequency of OT-induced uterine contraction. OT is one of the longest known drugs to induce myometrial stimulation and has played a role in parturition through the years. The classical action of myometrial stimulation by OT involves phospholipase C activation with release of inositol triphosphate and diacylglycerol leading to a subsequent release of Ca^{2+} [24]. It is therefore possible that MTT augments the Ca^{2+}-releasing mechanisms of OT. This was further supported by the potentiation by MTT of KCl-induced tonic uterine contraction. Concentrated solutions of KCl have been proposed to stimulate tonic contractions of smooth muscles through depolarization and

opening of L-type Ca^{2+} channels, the total effect of these activities results in an increase in intracellular Ca^{2+} concentration [25]. Concentrated solutions of KCl have also been recently reported to activate RhoA kinase (ROCK) in the myometrium [26]. The effect on KCl therefore suggests partial augmentation of L-type Ca^{2+} channels as well as possible activation of ROCK in the uterine smooth muscle.

An attempt to investigate involvement of MTT with potassium channels and other receptors showed that in the presence of amiodarone, the stimulatory effect of MTT on OT was attenuated. Amiodarone is known to inhibit the human Ether-à-go-go-Related Gene (hERG)-encoded potassium channels as its primary activity [27,28] leading to repolarization and inhibition of contractility. This therefore suggests an additional inhibitory interaction of MTT on hERG potassium channels which may also play a role in the stimulatory effect of MTT. Glibenclamide which is known to inhibit adenosine triphosphate-sensitive K^+ channels (K_{ATP}) [29] did not alter the effect of MTT on OT-induced contractions. Similarly propranolol, a β-adrenergic receptor agonist which inhibits uterine contractility via activation of K_{ATP} channels [30], did not alter the stimulatory effect of MTT on OT-induced uterine contractility. Taken together, it would seem that MTT preferentially affects specific subtypes of potassium channels in the myometrium. The potassium ion channels are among the ion channels that play major roles in myometrial activity as they are central to the regulation of the ion permeability across the myometrial membrane [31]. Opening of these channels as occurs with amiodarone causes an outward flow of K^+ and subsequently a reduction in excitability and contractility [32]. There are different types of K^+ channels among them are the K_{ATP} channels and the hERG channels. These channels differ in their molecular structure due to different expressions of the rectifying potassium families [33]. These differences create distinct pharmacological responses and sensitivities [33]. While the hERG channels reduce the outward flow of potassium during repolarization [34], K_{ATP} channels increase the outward flow of K^+ [31]. These effects might explain the differences in the effect of amiodarone and glibenclamide on the activity of MTT and also provide a knowledge-based hypothesis for further investigations on MTT. It is also important to note that though amiodarone has additional ion-channel blocking effects as well as inhibition of the β-adrenergic receptor [35,36], the inability of propranolol to abolish the stimulatory effect of MTT, knocks off the possible involvement of β-adrenergic activity.

High resolution mass spectrometric analysis of MTT revealed the presence of 12 compounds significantly present in MTT. Of these twelve, 8 of them were successfully identified while 4 remain unknown and may suggest novel compounds which further purification, spectrometric and spectroscopic analysis may assist in their characterization and elucidation. Importantly, the compounds identified were highly polar which is not surprising judging by the high water content of the leaves of *T. triangulare* [37]. The following classes of secondary metabolites were found: alkaloids, aliphatic acyclic compounds, cyclic alcohols, lipids, steroids and polyketides. A closer look at the pharmacological effects reported so far for the identified compounds are briefly described. Hydroxypyridine has been reported to reduce inflammation of the uterus [38]. However, vitamin B_6 a hydroxypyridine derivative [39] has been reported to have insignificant effect on preterm birth [40] and instead may increase the risk of preterm birth [41]. On the other hand, nifedipine also a hydroxypyridine derivative [42] is a well-known calcium-channel blocker used as a tocolytic remedy [43]. Based on this seeming conflicting reports, further investigation is therefore required into the role of 17-hydroxypyridine in MTT on uterine contractility. Citric acid was also identified in MTT but there appears to be no report on the effect of citric acid on uterine contractility. Studies are therefore required to investigate the potential benefits that may be associated with citric acid on female reproductive health. Nonetheless, citric acid has been identified as a lactogenesis marker after preterm delivery [44], in addition citric acid has been reported to exert significant antimicrobial activity [45] and may thus influence infection-induced preterm birth. Caffeoylquinic acid another compound identified in MTT has been reported to inhibit angiotensin-converting enzyme [46]. Other studies report anti-inflammatory effects of caffeoylquinic acid [47,48]. Angiotensin I has been reported to be a potent constrictor of uterine arteries [49] while angiotensin II has been reported to induce uterine contraction [50]. Taken together, inhibition of angiotensin converting enzyme by caffeoylquinic acid may promote uterine stimulation and may explain in part the effect of MTT observed in this study. The presence of hydroxyingenol and ferulic acid derivative in MTT suggests that the plant may be high in phytoestrogens. Ferulic acid has been reported to inhibit uterine contraction by acting on the oxytocin-receptor system [51],

similarly terpenoids such as hydroxyingenol has been reported to inhibit uterine contractions [52]. Phaeophorbide a was also identified in MTT and emerging roles of chlorophyll derivatives on uterine contractility are being mapped out. Phaeophorbide a from *F. exasperata* was earlier reported to decrease the amplitude of uterine contraction [53] and lipids such as octadecenoic acid also detected in MTT have been reported to inhibit uterine contractility [53]. The interaction of these different metabolites may also explain the dual effect of MTT observed on uterine contractility.

In conclusion, novel data has been reported in this study showing the leaves of *Talinum triangulare* to exert a biphasic effect on spontaneous uterine contraction. An effect that may be a reflection of varying receptor activities or a result of the varying constituents present in the leaves. It was also shown in this study that the plant may have selective effect on specific potassium channels in the myometrium which may also contribute to its effect. The use of the plant as a tocolytic may therefore depend on the concentration ingested and care must therefore be taken on consumption. This study also identified some phytochemical constituents which may play roles in the regulation of uterine contractility. Further studies are therefore required to investigate direct effects of these constituents on uterine contractility in order to promote the use of this plant as a sustainable and cheap alternative in the management of female reproductive health issues. It is also important to resolve the exact doses at which inhibition and/or stimulation occurs.

Author Disclosure Statement

The authors declare no conflict of interest.

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REFERENCES

[1] Georgiou HM, Di Quinzio MKW, Permezel M, Brennecke SP. Predicting Preterm Labour: Current Status and Future Prospects. Dis Markers 2015;2015:1–9. doi:10.1155/2015/435014.

[2] WHO. Born too soon: : the global action report on preterm birth. vol. 25 Suppl 1. 2012. doi:10.1002/yd.20044.

[3] Ezekwe MO, Besong SA, Igbokwe PE. Beneficial influence of purslane and waterleaf supplement to Human. FASEB J 2001;16:A639.

[4] Swarna J, Ravindhran R, Lokeswari TS. Characterization of Talinum triangulare (Jacq.) Willd. germplasm using molecular descriptors. South African J Bot 2015;97:59–68. doi:10.1016/j.sajb.2014.12.012.

[5] De Oliveira Amorim AP, De Carvalho AR, Lopes NP, Castro RN, De Oliveira MCC, De Carvalho MG. Chemical compounds isolated from Talinum triangulare (Portulacaceae). Food Chem 2014;160:204–8. doi:10.1016/j.foodchem.2014.01.114.

[6] Adeyemi O, Oyeniyi O, Mbagwu H, Jackson C. Evaluation of the gastrointestinal activity of the aqueous root extracts of Talinum triangulare. J Appl Pharm Sci 2011;3:61–7.

[7] Liang D, Zhou Q, Gong W, Wang Y, Nie Z, He H, et al. Studies on the antioxidant and hepatoprotective activities of polysaccharides from Talinum triangulare. J Ethnopharmacol 2011;136:316–21. doi:10.1016/j.jep.2011.04.047.

[8] Ravindra BP, Rama RD, Prasada RM, Krishna kanth J V., Srinivasulu M, Hareesh V. Hypoglycemic Activity of Methanolic Extract of Talinum Triagulare Leaves in Normal and Streptozotocin Induced Diabetic Rats. J Appl Pharm Sci 2012;2:197–201.

[9] Liao DY, Chai YC, Wang SH, Chen CW, Tsai MS. Antioxidant activities and contents of flavonoids and phenolic acids of Talinum triangulare extracts and their immunomodulatory effects. J Food Drug Anal 2015;23:294–302.

[10] Onwurah NN, Eke IG, Anaga AO. Antiulcer Properties Of Aqueous Extract Of Talinum Triangulare Leaves In Experimentally Induced Gastric Ulceration In Mice. Asian J Pharm Biol Res 2013;3:4–7.

[11] Ezekwe CI, Uzomba C, Okechukwu PCU. The Effect of Methanol Extract of Talinum Triangulare (Water Leaf) on the Hematology and Some Liver Parameters of Experimental Rats. Glob J Biotechnol Biochem 2013;8:51–60.

[12] Caunii A, Pribac G, Grozea I, Gaitin D, Samfira I. Design of optimal solvent for extraction of bio--active ingredients from six varieties of Medicago sativa. Chem Cent J

2012;6:123. doi:10.1186/1752-153X-6-123.
[13] Kota SK, Gayatri K, Jammula S, Meher LK, Kota SK, Krishna SVS, et al. Fetal endocrinology. Indian J Endocrinol Metab 2013;17:568–79. doi:10.4103/2230-8210.113722.
[14] Snegovskikh V, Park JS, Norwitz ER. Endocrinology of parturition. Endocrinol Metab Clin North Am 2006;35:173–91. doi:10.1016/j.ecl.2005.09.012.
[15] Kessner D, Chambers M, Burke R, Agus D, Mallick P. ProteoWizard: Open source software for rapid proteomics tools development. Bioinformatics 2008;24:2534–6. doi:10.1093/bioinformatics/btn323.
[16] Pluskal T, Castillo S, Villar-Briones A, Oresic M. MZmine 2: modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data. BMC Bioinformatics 2010;11:395. doi:10.1186/1471-2105-11-395.
[17] Macintyre L, Zhang T, Viegelmann C, Juarez Martinez I, Cheng C, Dowdells C, Abdelmohsen UR, Gernert C, Hentschel U, and Edrada-Ebel R. Metabolomic tools for secondary metabolite discovery from marine Symbionts. Mar Drugs. 2014 ;12(6); 3416-48.
[18] Yoccoz NG. Use, Overuse and Misuse of Significance Tests in Evolutionary Biology and Ecology. Bull Ecol Soc Am 1991;72:106–11. doi:10.2307/20167258.
[19] Nakagawa S, Cuthill IC. Effect size, confidence interval and statistical significance: A practical guide for biologists. Biol Rev 2007;82:591–605. doi:10.1111/j.1469-185X.2007.00027.x.
[20] Parks DH, Beiko RG. Identifying biologically relevant differences between metagenomic communities. Bioinformatics 2010;26:715–21. doi:10.1093/bioinformatics/btq041.
[21] Tzavara ET, Wade M, Nomikos GG. Biphasic effects of cannabinoids on acetylcholine release in the hippocampus: site and mechanism of action. J Neurosci 2003;23:9374–84. doi:23/28/9374 [pii].
[22] Szabadi E. A model of two functionally antagonistic receptor populations activated by the same agonist. J Theor Biol 1977;69:101–12. doi:10.1016/0022-5193(77)90390-3.
[23] Calabrese EJ. Hormesis and medicine. Br J Clin Pharmacol 2008;66. doi:10.1111/j.1365-2125.2008.03243.x.
[24] Wray S. Insights into the uterus. Exp Physiol 2007;92:621–31. doi:10.1113/expphysiol.2007.038125.
[25] Ratz PH, Berg KM, Urban NH, Miner AS. Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. Am J Physiol Cell Physiol 2005;288:C769–83. doi:10.1152/ajpcell.00529.2004.
[26] Kupittayanant S, Burdyga T, Wray S. The effects of inhibiting Rho-associated kinase with Y-27632 on force and intracellular calcium in human myometrium. Pflugers Arch Eur J Physiol 2001;443:112–4. doi:10.1007/s004240100668.
[27] Kiehn J, Thomas D, Karle C a, Schöls W, Kübler W. Inhibitory effects of the class III antiarrhythmic drug amiodarone on cloned HERG potassium channels. Naunyn Schmiedebergs Arch Pharmacol 1999;359:212–9. doi:10.1007/PL00005344.
[28] Zhang YH, Cheng H, Alexeenko VA, Dempsey CE, Hancox JC. Characterization of recombinant hERG K+ channel inhibition by the active metabolite of amiodarone desethyl-amiodarone. J Electrocardiol 2010;43:440–8. doi:10.1016/j.jelectrocard.2010.04.007.
[29] Li DL, Ma ZY, Fu ZJ, Ling MY, Yan CZ, Zhang Y. Glibenclamide decreases ATP-induced intracellular calcium transient elevation via inhibiting reactive oxygen species and mitochondrial activity in macrophages. PLoS One 2014;9. doi:10.1371/journal.pone.0089083.
[30] Lovasz N, Koncz A, Domokos D, Gaspar R, Falkay G. ATP-sensitive potassium channels modulate in vitro tocolytic effects of β2-adrenergic receptor agonists on uterine muscle rings in rats in early but not in late pregnancy. Croat Med J 2015;56:114–8. doi:10.3325/cmj.2015.56.114.
[31] Wray S. Uterine contraction and physiological mechanisms of modulation. Am J Physiol 1993;264:C1–18. doi:10.1016/S0140-6736(01)00669-9.
[32] Khan RN, Matharoo-Ball B, Arulkumaran S, Ashford ML. Potassium channels in the human myometrium. Exp Physiol 2001;86:255–64.
[33] Inagaki N, Seino S. ATP-sensitive potassium channels: structures, functions, and pathophysiology. Jpn J Physiol 1998;48:397–412.

[34] Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. Cell 1995;80:795–803. doi:10.1016/0092-8674(95)90358-5.

[35] Kodama I, Kamiya K, Toyama J. Cellular electropharmacology of amiodarone. Cardiovasc Res 1997;35:13–29. doi:10.1016/S0008-6363(97)00114-4.

[36] Doggrell SA. Amiodarone - Waxed and waned and waxed again. Expert Opin Pharmacother 2001;2:1877–90. doi:http://dx.doi.org/10.1517/14656566.2.11.1877.

[37] Akinnibosun FI, Adeola MO. Quality Assessment and Proximate Analysis of Amaranthus hybridus, Celosia argentea and Talinum triangulare obtained from open Markets in Benin City, Nigeria. J Appl Sci Environ Manag 2015;19:727–34.

[38] Volchegorskii IA, Mester KM. [Effects of 3-hydroxypyridine and succinic acid derivatives on the dynamics of dorsalgia and affective disorders after surgical treatment of disc herniation]. Eksp Klin Farmakol 2010;73:33–9.

[39] Kiruba GSM, Wong MW. Tautomeric equilibria of pyridoxal-5'-phosphate (vitamin B6) and 3-hydroxypyridine derivatives: A theoretical study of solvation effects. J Org Chem 2003;68:2874–81. doi:10.1021/jo0266792.

[40] Dror DK, Allen LH. Interventions with Vitamins B6, B12 and C in pregnancy. Paediatr Perinat Epidemiol 2012;26:55–74. doi:10.1111/j.1365-3016.2012.01277.x.

[41] Ronnenberg AG, Goldman MB, Chen D, W Aitken I, C Willett W, Selhub J, et al. Preconception homocysteine and B vitamin status and birth outcomes in Chinese women. Am J Clin Nutr 2002;76:1385–91.

[42] Elzenga JTM, Staal M, Prins HBA. Calcium-calmodulin signalling is involved in light-induced acidification by epidermal leaf cells of pea, Pisum sativum L. J Exp Bot 1997;48:2055–61. doi:10.1093/jxb/48.12.2055.

[43] Conde-Agudelo A, Romero R, Kusanovic JP. Nifedipine in the management of preterm labor: A systematic review and metaanalysis. Am J Obstet Gynecol 2011;204. doi:10.1016/j.ajog.2010.11.038.

[44] Cregan MD, De Mello TR, Kershaw D, McDougall K, Hartmann PE. Initiation of lactation in women after preterm delivery. Acta Obstet Gynecol Scand 2002;81:870–7. doi:aog810913 [pii].

[45] Nikawa H, Igarashi S, Takasu O, Tataka H, Harano F, Makihira S, et al. Chewing Gum Containing Citric Acid Reduces the Burden of Periodontal Pathogens. Open Food Sci J 2008;2:29–37.

[46] Oh H, Kang DG, Lee S, Lee HS. Angiotensin converting enzyme inhibitors from Cuscuta japonica Choisy. J Ethnopharmacol 2002;83:105–8. doi:10.1016/S0378-8741(02)00216-7.

[47] Kimura Y, Okuda H, Okuda T, Hatano T, Arichi S. Studies on the activities of tannins and related compounds, X. Effects of caffeetannins and related compounds on arachidonate metabolism in human polymorphonuclear leukocytes. J Nat Prod 2004;50:392–9. doi:10.1021/np50051a009.

[48] Peluso G, De Feo V, De Simone F, Bresciano E, Vuotto ML. Studies on the inhibitory effects of caffeoylquinic acids on monocyte migration and superoxide ion production. J Nat Prod 1995;58:639–46. doi:Doi 10.1021/Np50119a001.

[49] Hannan RE, Davis E a, Widdop RE. Functional role of angiotensin II AT2 receptor in modulation of AT1 receptor-mediated contraction in rat uterine artery: involvement of bradykinin and nitric oxide. Br J Pharmacol 2003;140:987–95. doi:10.1038/sj.bjp.0705484.

[50] Lalanne C, Mironneau C, Mironneau J, Savineau JP. Contractions of rat uterine smooth muscle induced by acetylcholine and angiotensin II in Ca2+-free medium. Br J Pharmacol 1984;81:317–26.

[51] Ozaki Y. Antiinflammatory effect of tetramethylpyrazine and ferulic acid. Chem Pharm Bull (Tokyo) 1992;40:954–6.

[52] Devappa RK, Makkar HPS, Becker K. Jatropha diterpenes: A review. JAOCS, J Am Oil Chem Soc 2011;88:301–22. doi:10.1007/s11746-010-1720-9.

[53] Bafor EE, Lim C V., Rowan EG, Edrada-Ebel R. The leaves of Ficus exasperata Vahl (Moraceae) generates uterine active chemical constituents. J Ethnopharmacol 2013;145:803–12.