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Research article

Ex-vivo Uterine Contractile activities of Leaf Extract and Fractions of *Manniophyton fulvum* Mull. Arg. (Euphorbiaceae) and Identification of Associated Secondary Metabolites

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ABSTRACT

Manniophyton fulvum is a herb with claimed anti-dysmenorrhea properties ascribed to the leaves in ethnomedicine. This study aimed to determine the effects of the methanol leaf extract and fractions on isolated mouse uterine contraction**.** Methanol extract of the leaf was obtained by Soxhlet extraction and partitioned into chloroform and water. The isolated and mounted mouse uterine tissues was treated with the extract, chloroform and aqueous fractions at a concentration range of 0.0007 – 7.777 µg/ml. The chloroform and aqueous fractions were equally tested on oxytocin and potassium chloride pre- contracted uterine tissue at concentration range of 0.34 – 34.3 µg/mL. LC-HRFTMS analysis of methanol extract and aqueous fraction was also carried out. The extract and aqueous fraction were observed to significantly ($p< 0.05$) increase the frequency and amplitude of spontaneous contractions in a concentration dependent manner, while the chloroform fraction concentration-dependently inhibited similar contractions. The aqueous fraction potentiated oxytocin and potassium chloride augmented contractions in a similar manner, while the chloroform fraction significantly inhibited augmented contractions. Spectroscopic analysis identified eight and nine compounds in the methanol extract and aqueous fraction respectively, belonging to coumarin, terpenoid, and flavonoid classes of natural compounds. Evidence from this study suggests that constituents responsible for uterine contraction are present in the polar (aqueous) fraction of the extract, while the non-polar fraction (chloroform) contains compounds with uterine relaxing effects.

Keywords: *Manniophyton fulvum, Euphorbiaceae, HRFTMS, Dysmenorrhea, Preterm birth/labour*

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INTRODUCTION

Dysmenorrhea, also known as painful menses, is characterized by cramps in the lower abdomen, pelvic and back pain in women during menses. Pain may be accompanied by nausea, vomiting, diarrhea, headache, and general discomfort (Bernardi *et al.*, 2017). Primary dysmenorrhea (PD) describes the condition when there is no associated pathological pelvic condition, while in secondary dysmenorrhea (SD), an identifiable pathological condition such as endometriosis, fibroids, adenomyosis, and pelvic inflammatory disease may be present (Proctor and Farguhar, 2006). PD, a leading cause of recurrent short-term absenteeism from school and work is highest in women of reproductive age particularly; in adolescents with a reported prevalence of 20 to 90 percent. It equally imposes considerable limitations on social and recreational activities, quality of sleep, and overall quality of life (Sharma, 2008; MacGregor *et al*., 2023). Risk factors for PD include obesity, early menarche (before the age of 12), family history, heavy/prolonged menstrual bleeding, stress, depression, anxiety, and a history of use of tobacco (Sultan *et al*., 2012). Although the exact mechanisms of PD have not been fully elucidated, the uterine smooth muscle dysfunction and pain observed have been linked to high levels of uterine prostaglandins (PGs), serum vasopressin, leukotrienes, and estradiol (Dawood, 2006). Pharmaco-therapeutic options for the management of PD include the non-steroidal anti-inflammatory drugs (NSAIDs) and the combined oral contraceptive pills. However, NSAIDs are effective in only 66 percent of women (Proctor and Farquhar, 2006) suggesting that there remains a percentage of women, in whom they have proved either refractory or intolerable (Oladosu *et al*., 2017). Identifiable gastrointestinal, neurological, and cardiovascular

side effects from the use of NSAIDs have equally limited their use in the management of PD (Majoribanks *et al.,* 2015). The combined oral contraceptive pill (OCP) is associated with side effects such as nausea, headaches, weight gain, and contraindication in women of reproductive age thus limiting their use (Wong *et al*., 2009).

Mechanisms responsible for PD have been implicated in preterm labor (Pl) which results in preterm birth (PB); which is neonatal deliveries before 37 weeks or 259 days of gestation (Beck *et al.,* 2010). Babies born preterm have higher chance of dying or suffering long-term adverse health conditions such as cerebral palsy, mental retardation, heart, and visual impairments, and overall poor health and growth. Progressing to adulthood, they present with increased chances of developing cardiovascular, cerebrovascular, chronic renal, pulmonary, and metabolic disorders (Luu *et al*., 2016). Tocolytics, promote quiescence of the uterus and are used to prevent and manage Pl. They include; beta-adrenoceptor agonists (terbutaline), calcium channel blockers (nifedipine), magnesium sulfate, and NSAIDs (indomethacin) (Patel and Ludmir, 2019). These are linked with side effects such as; renal failure, gastrointestinal bleeding (indomethacin), transient hypotension (nifedipine), muscular paralysis, maternal tetany respiratory depression (magnesium sulfate), myocardial ischemia, hypokalemia, and hyperglycemia (terbutaline), raising safety concerns for their continued use. Oxytocic agents are used as adjuvants to stimulate and sustain uterine contractions and aid in successful neonatal delivery in cases of post-term pregnancy, lack of progress, and suspected intrauterine growth restriction (Mozurkewich *et al.,* 2009). They are also used in the management of post-partum bleeding, but have associated side effects such as nausea, vomiting, arrhythmias, severe weakness, and excessive bleeding (Kabilan, 2014).

Medicinal plants hold the promise of an important source of new drug entities with the possibility of better therapeutic action and fewer side effects as some of them have been used in ethnomedicine to manage PD and PL. *Foeniculum vulgare*, *Mentha piperita*, *Cinnamomum zeylanicum*, *Zataria multiflora*, *Zingiber officinale*, and *Cuminum cyminum* are examples of such plants (Mirabia *et al*., 2014).

Manniophyton fulvum Mull Arg; a member of the Euphorbiaceae family is well recognized for its diverse usefulness in medicine, nutrition, and the economy. It is a deciduous shrub or climber that can grow up to 30- 40 meters long (Burkill, 1994). Ethno-medicinally, the root, stem bark, and leaves are used for their analgesic property. They are also used against diarrhea, cough, stomachache and, bronchitis. Sap from the stem is used to treat wounds, while that from the leaf is used against ear and dental problems. In Congo (Brazzaville), Cote d Ivoire and Nigeria, the leaf extract is used against dysentery, hemoptysis and dysmenorrhea. Leaf decoctions are also used in cases of inflammation while the seeds are used against hemorrhoids and blood disorders (Burkill, 1994). Antioxidant, anti-inflammatory (Nia et al., 2005), anti-diabetic (Onyemairo *et al.,* 2015), antidiarrheal (Ojieh *et al*., 2013) and aphrodisiac properties (Emudainohwo *et al*., 2010) have been reported for the plant. It is commonly known as "Gasso nut" and called "Ebumen" by the Binis; "Gbogbo – nigiri" by the Ibos "Iken-iken" by the Anangs (Burkill, 1994; Omokhafe *et al.,* 2012). Based on the claimed effects of the leaf of the plant on the uterine contraction, this study investigated the uterine contraction modulatory effects of the methanol leaf extract and fractions on the isolated mouse uterus. Spectroscopic identification of compounds present in the extract and fraction was also carried out.

MATERIALS AND METHODS

Chemicals and Reagents: All reagents and chemicals used were of analytical grade and procured from various sources. Oxytocin and potassium chloride were obtained from Sigma Aldrich (England), while all the salts used in preparing the physiological solution were obtained from British Drug House (BDH) Chemical Limited, (England).

Plant collection, preparation, and extraction: The whole plant of *M. fulvum* was collected in Iwu town, Ovia North East Local Government Area of Edo state Nigeria, situated between latitudes 5º40' and 7º40'North and longitudes 5º00' and 6º30'East. (Akinbo and Okaka, 2010). Identification and authentication of the plant were carried out by Dr. A. Oseyemi, of the Plant Herbarium Unit, Forestry Research Institute of Nigeria (FRIN) Ibadan, Nigeria. A voucher specimen with the number FH1109931, was curated at the institute. The leaves were separated from the stem, freed of debris, shade dried for 72 hrs, and thereafter in an oven at 40ºC for 30 mins. Dried leaves were reduced to smaller pieces by crushing with a gloved hand and to a fine powder in a milling machine (Christy Norris, England). Powdered plant material (620 g) was extracted with methanol (2.5 L) in a Soxhlet apparatus and extract obtained was filtered through a Whatman filter paper (Hillsboro, USA) and concentrated under vacuum (40ºC). Further drying of the extract was achieved in an oven (Carbolite, United Kingdom) at 40ºC to obtain the methanol extract (MMF; 63.53 g), preserved at 4ºC till needed for further use.

Fractionation of the methanol extract: Fractionation of MMF was carried out according to the method of Egua *et al*., (2014) with a slight modification. A weighed amount of extract (45 g) was dissolved in 200 mL of warm distilled water (20ºC), allowed to cool to ambient temperature, and partitioned with chloroform (400 mL) in100 mL succession. Fractions were pooled, concentrated under vacuum to yield the chloroform fraction (CMF; 13.70 g). The residual aqueous fraction was equally concentrated to obtain the aqueous fraction (AMF; 25.07 g). Each fraction was preserved at 4ºC until required.

Animals: Matured non-pregnant female albino mice (5 – 6 months), weighing 25 – 30 g, housed in polypropylene cages in the animal house of the Department of Pharmacology, Faculty of Pharmacy, University of Benin were used for the experiments. They were allowed to acclimatize for two weeks before use. Housing conditions for animals included; environmentally controlled room temperature of 28ºC ± 5ºC, relative humidity of 70-75 ± 3%, natural light and dark cycles of approximately12 hrs day/ night, and free access to rat chow and clean water. Ethical approval for the study was obtained

from the Ethics committee of the Faculty of Pharmacy, University of Benin vide reference EC/FP/019/17. The animals were handled as much as possible in line with standards of the Public Health Service Policy on Humane Care and Use of Laboratory Animals (NIH, 2015).

Contractility studies

Preparation of Uterine Tissue: Four animals per protocol were used in each set of experiments. Animals were administered diethylstilbestrol (DES) orally at a dose of 1.0 mg/kg 24 hrs before experiments and animals in oestrus as determined by vaginal smear obtained according to the method of Caligioni (2009) were humanely sacrificed by cervical dislocation. The uterine horns were excised, freed from connective tissues, and placed in previously warmed and aerated De Jalon's physiological solution. The uterine tissue was transected medially to obtain lengths of approximately 1-2 mm segments and mounted in a warmed 10 mL organ bath maintained at 37ºC containing continuously aerated De Jalon's physiological solution (Bafor et al., 2016). A tension of 0.7 g was applied and tissue was allowed to equilibrate for 30 mins to obtain regular spontaneous contractions. The force and frequency of uterine contraction in the muscle layers were measured with a 7003 E-isometric force transducer (Ugo Basile, Varise, Italy) connected to a17 400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile).

Evaluation of the effect on spontaneous contraction: The cumulative effect of MMF, CMF, and AMF on uterine smooth muscle contractility was determined at a concentration range of 0.0007 – 7.777 µg/mL. A contact time of 5 mins was allowed following each concentration of extract/fraction administered. Further experiments were performed with the fractions only.

Evaluation of the effect of CMF and AMF on oxytocin-induced uterine contraction: The effect of fractions on oxytocin (OT) induced uterine contraction was investigated by initially determining a concentration-response to oxytocin (0.006 – 88.86 pg/mL) in the absence of AMF and CMF. This was then repeated in separate experiments in the presence of the fractions at 3.43 and 34.3 µg/mL for CMF and 0.034 - 34.3 µg/mL for AMF, added cumulatively to the bath.

Determination of the effect of CMF and AMF on potassium chloride-induced uterine contraction: The effect of CMF and AMF in the presence of potassium chloride (KCl) on uterine contraction was determined by adding KCl (20 mM) to the bath containing the uterine tissue, and without washing, cumulative concentrations of the fractions (0.034 – 34.3 µg/mL) were determined in separate experiments.

Liquid chromatography – High-resolution mass spectrometry identification of Constituents in MMF and AMF: Liquid chromatography- High-resolution Fourier Transform mass spectrometry (LC-HRFTMS) analysis of MMF and AMF was performed on a Dionex Ultimate-3000 (DIONEX, Sunnyvale, CA, USA) coupled to a Thermo Scientific Exactive Orbitrap system (Thermo Fisher Scientific (Bremen) GmbH, Bremen, Germany. Mass accuracy was set to less than 3.0 ppm and the instrument was externally calibrated according to the manufacturer's instruction before the run. Solvent blank was prepared without extract, while samples were prepared at a concentration of 1 mg/Ml in 80: 20 methanol: dichloromethane (MeOH: DCM). Other parameters used were as previously described by Bafor *et al* (2018a). Chemical formulae of peaks generated from the run were predicted using the formula prediction tool developed by MZmine. ChemBiofinder version 13 (Perkin Elmer Informatics, Cambridge UK) to access hits from the database.

Data analysis: Mean frequency and amplitude of contraction were computed from contractions occurring at the last 3 mins of the phasic response. Graphpad Prism, Version 7.03 (Graphpad Software Inc, San Diego CA, USA) was used in analyzing results. Data are expressed as mean ± standard error of the mean (SEM) where $n = 4$. One-way analysis of variance was carried out with Dunnett's post hoc test and t-test where applicable. P values of ≤ 0.05 were taken to represent the minimum level of significance.

Where data sets with sufficient data points were obtained, mean log concentration-response curves were analyzed by fitting data to a variable slope logistic equation using the equation values: Y =Bottom + (Top-Bottom)/(1 + 10^(Log IC_{50} -X)*Hillslope), where Y is the response that starts at the bottom and goes to the top in a sigmond shape, X is the logarithm of concentration and half-maximal inhibitory concentration (lC_{50}) half-maximal inhibitory concentration that gives a response halfway between bottom and top

RESULTS

Effect of extract and fractions on spontaneous uterine contraction: Spontaneous contractions by the isolated uterine tissue were observed as recorded in the Uni-recorder. Evaluation of the data indicated that MMF and AMF increased the amplitude and frequency of uterine contractions in a dose-dependent manner while CMF decreased the amplitude and frequency equally in a dose-related manner. These were found to be statistically significant at $*p < 0.05$ and $**p<0.01$ (Figure 1a and b). The increase in amplitude and frequency of contraction produced by AMF was, however, higher than that produced by MMF.

Effect of fractions on oxytocin-induced contraction: AMF was observed to evoke an increase in the amplitude and frequency of OT-induced uterine contractions in a dose-dependent manner that was statistically significant ($*p < 0.05$, $**p<0.01$) compared with control while CMF evoked a decrease in the frequency and amplitude of contraction in the presence of oxytocin. This effect was observed to be dose-dependent as shown in figures 2a, 2b and 2c).

Effect of fractions on Potassium chloride-induced contraction: AMF increased KCl-induced contraction at concentrations of 3.43 and 34.3 µg/ml while, CMF was observed to decrease KCl-induced contraction in a dose-dependent pattern. These results were statistically significant ($*p < 0.05$) at the concentration of 34.3µg/ml (figure 3a and 3b).

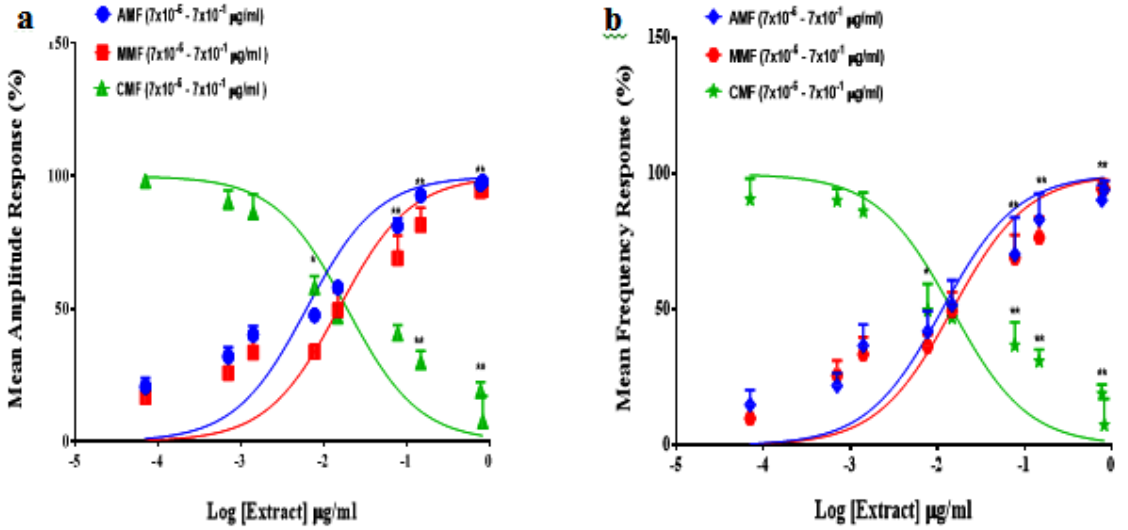

Figure 1:
Concentration-response curves showing the effect of **(a)** **MMF, AMF, and CMF** respectively on the amplitude of spontaneous uterine contractions and (**b**) Concentration-response curves showing the effect of **MMF, AMF, and CMF** on the frequency of spontaneous uterine contractions n =4

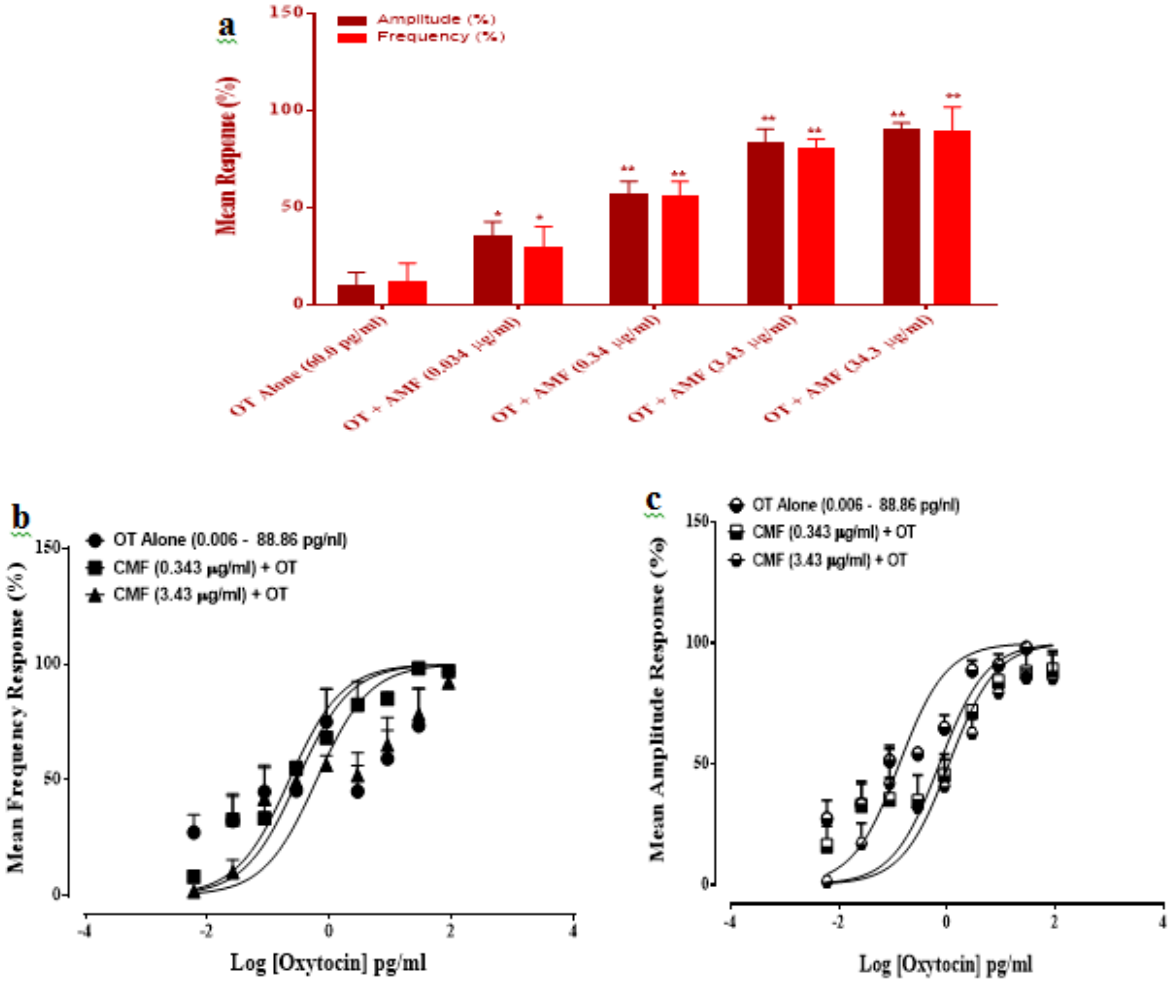

Figure 3
Cumulative effect of (**a) AMF** on high KCl-induced contractions (80 nM) and **(b)** Bar graphs showing the cumulative effect of **CMF** on high KCl-induced contractions (80 nM) n = 4

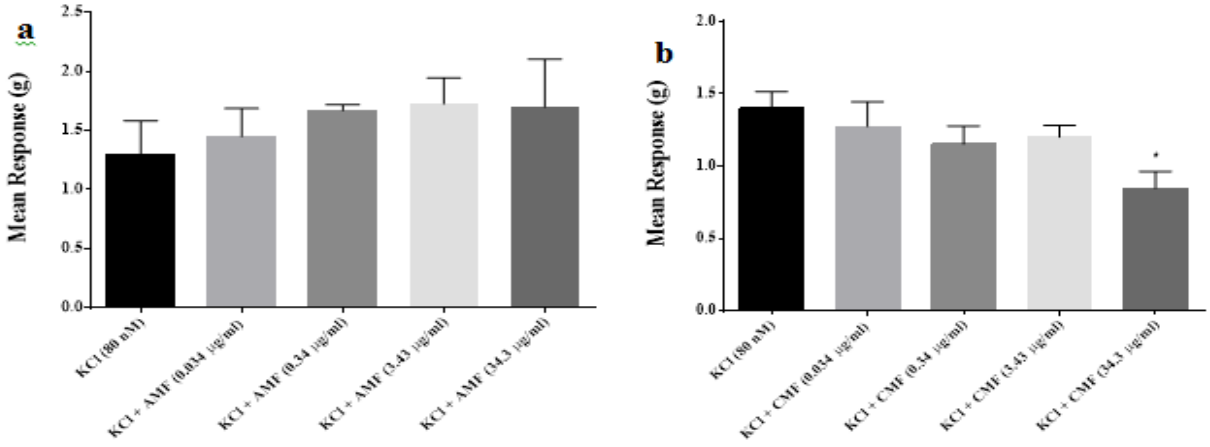

Figure 3:
Cumulative effect of (**a) AMF** on high KCl-induced contractions (80 nM) and **(b)** Bar graphs showing the cumulative effect of **CMF** on high KCl-induced contractions (80 nM) n = 4

Table 1.
Putatively identified and unidentified compounds in MMF

S/N	Compound Name	Molecular formula	Molecular weight (g/mol)	m/z	RT (min)
1	2,4-Diamino-2,4,6-trideoxygalactose	$C_{17}H_{24}N_2O_5$	336.1685	$[M+H]^+$337.1758	6.16
2.	2,4-Diamino-2,4,6-trideoxygalactose	$C_{19}H_{26}N_2O_6$	378.1794	-377.1721	10.31
3.	Cucurbita-5,24-diene-3,7,16-triol	$C_{36}H_{60}O_7$	604.4351	$[M+H]^+$605.4424	19.35
4.	N-Methyltyrosyl-N-methyltyrosylleucylalanine	$C_{29}H_{40}N_4O_7$	556.2911	$[M-H]^-$555.2838	21.15
5.	17-Hydroxyingenol	$C_{34}H_{40}O_9$	592.2690	$[M+H]^+$609.2711	28.57
6.	2(3,20)-Abeo-2,5,7,10,13-pentahydroxy-4(20),11-taxadien-9-one	$C_{35}H_{42}O_{10}$	622.2796	$[M+H]^+$623.2869	31.15
7.	Phaeophorbide a	$C_{36}H_{38}N_4O_5$	606.2848	$[M+H]^+$607.2921	32.13
8.	Bidenphytin B	$C_{55}H_{74}N_4O_6$	886.5607	$[M+H]^+$887.5679	32.81

Unidentified compounds in MMF

	Predicted molecular formula	Double bond equivalence	Molecular weight (g/mol)	m/z	RT (min)
9.	$C_{18}H_{22}N_2O_3$	9.0	314.1629	$[M+H]^+$315.1702	10.14
10.	$C_{34}H_{58}O_{16}$	6.0	722.3717	$[M-H]^-$721.3644	17.14
11.	$C_{34}H_{36}N_5O_4$	19.5	578.2756	$[M-H]^-$577.2683	17.73
12.	$C_{27}H_{42}N_7O_6$ $C_{28}H_{48}O_{11}$	10.5 5.0	560.3193	$[M-H]^-$559.3121	19.40
13.	$C_{20}H_{43}N$	0.0	297.3395	$[M+H]^+$298.3468	24.30
14.	$C_{32}H_{38}O_7$ $C_{33}H_{34}N_4O_3$	14.0 19.0	534.2635	$[M+H]^+$535.2708	30.93
15.	$C_{55}H_{74}N_4O_6$ $C_{60}H_{74}N_2O_4$	21.0 25.0	886.5614	$[M+H]^+$887.5687	31.19

Double bond equivalence indicates the number of rings and double bonds in the structure, where 1 ring = 1 double bond equivalence.

HPLC-MS Analysis: Spectroscopic analyses of MMF lead to the identification of eight compounds belonging to different phytochemical groups, while 15 compounds could not be unidentifiable. Equally, nine compounds were identified in AMF and eleven compounds were unidentifiable (Tables 1 and 2).

DISCUSSION

Generally, medicinal plants and other agents that affect myometrial contractions do so by altering the chemical properties, excitability, and/or conductivity of uterine smooth muscle cells to Ca^{2+} and K^+ (Garfield and Maner, 2007).

Uterine contractions are associated with membrane-mediated changes that lead to an increase in cytoplasmic Ca^{2+} concentration. Studies have shown that the influx of extracellular Ca^{2+} and release of Ca^{2+} from intracellular spaces with its subsequent accumulation in the cytoplasmic space, result in increased contractions (Gruber and O'Brien, 2011). A process initiated by the coupling of Ca^{2+} with the protein, calmodulin. The Ca^{2+} - calmodulin complex activates myosin light (MLC) kinase, which in turn phosphorylates MLC. Conformational changes induced in myosin leads to an increase in frequency and amplitude of contraction (Gruber and O'Brien, 2011).

Table 2.
Putatively identified and unidentified compounds in AMF

S/N	Compound Name	Molecular formula	Molecular weight (g/mol)	m/z	RT (min)
1	2,4(1H,3H)-Pteridinedione	$C_8H_8N_4O_2$	192.0650	$[M-H]^-$191.0577	1.07
2.	2,4-Furandicarboxylic acid	$C_7H_6O_5$	170.0212	$[M-H]^-$169.0140	2.20
3.	Brevifolincarboxylic acid	$C_{13}H_8O_8$	292.0216	$[M-H]^-$291.0143	4.13
4.	Aconitic acid	$C_8H_8O_5$	184.0369	$[M-H]^-$183.0297	4.29
5.	Chebulic acid	$C_{16}H_{14}O_{11}$	382.0535	$[M-H]^-$381.0462	4.93
6.	3-Acetyl-8-hydroxy-4-oxo-4H-1-benzopyran-2-carboxylic acid	$C_{12}H_8O_6$	248.0317	$[M-H]^-$247.0245	5.19
7.	2,5-Diaminopentanoic acid; (S)-form, 5-N-Benzyloxycarbonyl, 2-N-Ac, Me ester	$C_{16}H_{22}N_2O_5$	322.1526	$[M+H]^+$323.1599	5.43
8.	Ellagic acid	$C_{14}H_6O_8$	302.0058	$[M-H]^-$300.9986	5.75
9.	Deacetylalvaradoin E; 10-Epimer, 10-hydroxy, 5'-Ac	$C_{22}H_{22}O_{10}$	446.1210	$[M-H]^-$445.1138	6.21
Unidentified compounds in AMF					
	Predicted molecular formula	**Double bond equivalence**	**Molecular weight (g/mol)**	**m/z**	**RT (min)**
10.	$C_{14}H_{20}N_4O_8$	7.0	372.1295	$[M-H]^-$371.1222	1.13
11.	$C_{18}H_{12}N_5O_4$	15.5	362.0878	$[M-H]^-$361.0805	1.29
12.	$C_{27}H_{22}O_{18}$	17.0	634.0808	$[M-H]^-$633.0735	4.41
13.	$C_{30}H_{23}N_2O_{15}$	20.5	651.1074	652.1147	4.45
14.	$C_{29}H_{14}N_6O_{14}$ $C_{27}H_{12}N_9O_{13}$	26.0 26.5	670.0575	$[M-H]^-$669.0503	4.45
15.	$C_{28}H_{26}N_4O_{10}$	18.0	578.1637	$[M-H]^-$577.1564	6.01
16.	-	-	-	-	11.79
17.	-	-	-	-	14.74
18.	-	-	-	-	17.24
19.	-	-	-	-	21.82
20.	-	-	-	-	35.71

Double bond equivalence indicates the number of rings and double bonds in the structure, where 1 ring = 1 double bond equivalence, - = unknown

This process, mediated through receptor-operated channels (ROCs) results in observed contraction. Membrane depolarization caused by high K+ is mediated through voltage-operated channels (VOCs). It results in the increased efflux of K^+ and increased influx and sequestering of cytoplasmic Ca^{2+}. This results in increased smooth muscle excitability and contraction. In the present study, MMF and AMF potentiated the spontaneous ex-vivo contractions of the mouse uterus. They caused an increase in the force and amplitude of contraction, suggesting induction of increased influx and decreased efflux of cytosolic Ca^{2+} through ROCs and/or VOCs. The increase in contraction evoked by AMF was higher than that seen with MMF. Kupittayanant *et al*., (2014) reported the increase in frequency and amplitude of spontaneous contraction in the isolated rat uterus by seed extracts of *Punica granatum* and rhizomes of *Zingiber officinale*. CMF caused a decrease in the amplitude and force of spontaneous contraction in the mouse myometrium in a time and concentration-dependent manner, implying an inhibition of the operations of ROC and VOC.

The mouse uterus is populated with oxytocin receptors which when stimulated result in increased myometrial contraction due to increased influx of Ca2+, increased MLC phosphorylation, and prostaglandins synthesis. The action of OT occurs via various pathways including; coupling with G-protein, VOCs, and ROCs (Wray, 2014). In the present study, MMF and AMF augmented the contraction due to OT, while CMF inhibited it. These findings suggest an interference of the extract and fractions in one or more of the signaling pathways that led to observed effects. While it can be postulated, that AMF may have mobilized Ca2+ into the cytoplasm through any of the pathways proposed for the action of OT in the myometrium, CMF acted to inhibit that action.

Agents such as KCl induce myometrial contractions through VOCs, which cause Ca^{2+} channel activation and prevent membrane re-polarization, resulting in increased Ca^{2+} influx and observed contractions (Ratz et al., 2005). Any agent that blocks the mechanism of operation of these VOC channels will inhibit KCl-induced contraction in uterine smooth muscle. CMF inhibited the contraction due to KCl. The observed action could be due to the blocking of Ca^{2+} influx through membrane depolarization and/or VOCs. Similarly AMF increased the contractions due to KCl by either by stimulating the VOCs channels, facilitating Ca^{2+} influx and/or induction of membrane depolarization.

Conceivably, the methanol leaf extract and fractions of *M. fulvum* possessed different chemical components, some of which were identified by spectroscopic analysis. Cucurbita-5, 24-diene-3,7,16-triol, a triterpenoid, previously identified in several species of the genus mormodica including *Mormodica charantia* (Jai *et al*., 2017) was identified in MMF. Though reports of its effect on uterine contractions are absent at this time, previous studies have highlighted the inhibitory activity of different terpenoids on spontaneous and agonist-induced

myometrial contractions by varied mechanisms. Kaurenoic acid, a diterpene isolated from *Capaifera lonsdorfi* and 3-α-Angeloyloxy-2α-hydroxy-13-14-2-dehydrocativic acid from *Brikella paniculata* are examples of terpenoids with inhibitory activity on agonist and non-agonist induced myometrial contractions (de Alencar *et al*., 2003; Ponce-Monter *et al*., 2007). Phaeoporbide A and Bidenphytin B are products of chlorophyll metabolism in plants. They reportedly induce uterine smooth muscle relaxation by inhibiting phosphoinositol (IP3) and up-regulating cAMP (Bafor *et al*., 2018). Brevifolincarboxylic acid and ellagic acid are natural coumarins identified in AMF. They have not been associated with uterine smooth muscle activity at this time. However, natural coumarins, such as osthole is known to interfere with calcium influx and inhibit *in-vitro* smooth muscle contraction (Sadraei *et al*., 2012).

In conclusion, evidence from this study indicates that the methanol leaf extract of *M. fulvum* contains constituents with uterine smooth muscle stimulatory and inhibitory activities. However, dominance of constituents responsible for uterine stimulatory activity seems to be more as it was observed to augment spontaneous contraction of the uterus compared to the fractions. The increased frequency and amplitude of contractions observed due to AMF can be inferred to result from the removal of those constituents with uterine contraction inhibitory activity from MMF, following solvent-solvent partitioning. Equally, the uterine inhibitory activities observed with CMF suggest the preponderance of constituents able to interfere with pathways that, results in myometrial stimulation and contractions in the fraction. The potential of the leaf extract and fractions of *M. fulvum* in modulating uterine contraction is demonstrated in this study. Further studies are suggested to explore this potential to yield compounds of interest in the treatment of various conditions that affect or may involve contraction of the uterus.

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