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UTEROTONIC EFFECT OF METHANOL EXTRACT AND FRACTIONS OF *PSIDIUM GUAJAVA* L. (MYRTACEAE) STEM BARK ON FEMALE WHITE ALBINO RATS

Adebayo Anthony GBOLADE[1,*], Oluwasegun Adekanmi ADEDOKUN[1,2], Peace EVANS[1]

[,1]Phytotherapy Research Group, Department of Pharmacognosy, College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria*
[1,2]Department of Pharmacognosy, College of Pharmacy, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria

***Corresponding author's email**: gbolade.adebayo @iuokada.edu.ng**; Telephone**:+2348034709137, ORCID iD: 0000-0001-9627-840X

ABSTRACT

Background and aim: *Psidium guajava* L. (Myrtaceae), commonly known as the guava tree, has many medicinal properties which include anti-microbial, and inflammatory and healing properties, in addition to its folkloric usage in facilitating childbirth.
Methods: This study evaluated the effect of methanol (MeOH) crude extract, aqueous (AQ) and dichloromethane (DCM) fractions of *P. guajava* stem bark on isolated uterus of non-pregnant estrogenized female rats at varying doses to determine on uterine contractility in the spontaneous, oxytocin-induced and high KCL-induced contractions models, and also in calcium-free physiological salt solution.
Results: Concentration-dependent increases in amplitude and frequency of contractions were observed for extract and AQ fraction in the spontaneous contraction experiment. For the oxytocin pre-contracted uterus, only the fractions gave concentration-dependent decreases in contraction. Moreover, only extract and AQ fraction potentiated KCL-induced contraction at 10 mg/mL. In addition, all three samples at 10 mg/mL, increased amplitude of uterine contraction in calcium-free PSS by 15.25-15.41%.
Conclusion: Although not as active as oxytocin, crude MeOH extract of *P. guajava* was more potent than the two fractions in eliciting oxytocic effects on isolated rat uterus by a different mechanism, thereby Justifying the folkloric usage of *P. guajava* stem bark in inducing labour.

Keywords: *Psidium guajava,* stem bark, methanol extract, oxytocic effect, female rat uterus

INTRODUCTION

Guava, Psidium guajava L. (Myrtaceae) is a small tropical evergreen tree valued for its fruits attaining 10 m in height [1]. It is naturalized in many regions of the world including South America, South and West Africa, South East Asia, South India and Sri Lanka but now cultivated in other tropical and subtropical climates [1] for its nutritional purposes. Different extracts of *P. guajava are reported to possess* several biological activities, including antibacterial, antioxidant, antifungal, antimalarial, antitussive effects amongst others [2]. The plant is traditionally used to treat diabetes, diarrhoea, gastroenteritis, caries, stomach-ache, hypertension, toothache, vomiting, ulcers, inflamed gums, coughs, and malaria amongst others [2]. It is also listed as a folkloric recipe for inducing labour in Bayelsa State, Nigeria [3] and Cote d'Ivoire [4].

A review of literature on plants used to induce labour in Sub-Saharan Africa has been documented [5]. Collins *et al* [6] have published folkloric labour-inducing practices with medicinal plants at village level in Madagascar. In addition, a global review of ethnobotanical surveys of uterotonic plants in Africa has been published [7]. With reference to Nigeria, scattered reports abound on uterotonic effects of plants. Among the nine traditional uterotonic plants from the northern and South-South of Nigeria investigated at cellular level, *Commelina africana, Sida corymbosa, Duranta repens, Vernonia amygdalina* were found to be highly active [8]. In another study by Ijioma *et al* [9], only *Uvaria chamae,* among the six traditional labour-inducing plant components of an herbal formulation had contractile effect. Furthermore, bioactive oxytocic compounds have been isolated from only few Nigerian plants such as *Ficus exasperata* [10], while a global review of bioactive oxytocic compounds was earlier documented by Gruber and O'Brien [7].

Presently, the oxytocic potential of *P. guajava* is unknown. Therefore, as part of ongoing research endeavours to document scientifically investigated oxytocic plants in Nigeria, we investigated methanol extract and fractions of the stem bark of *P. guajava* for uterotonic effect in non-pregnant oestronized female white albino rats.

MATERIALS AND METHODS

***Plant collection and extraction*:** Stem bark peels of *P. guava* were harvested from trees growing in Idi-Ayunre Forest, Ibadan, Oyo State, Nigeria in January 2022, and authenticated (voucher no. IUO/10/013) at Department of Pharmacognosy, Igbinedion University Okada herbarium. Bark peels were cut into small pieces and air-dried for seven days on concrete floor, ground into coarse powder using a locally fabricated grinder. Powdered plant (350 g) was exhaustively macerated with methanol (MeOH), filtered, and extract concentrated on electric water bath (60^oC) to yield dried residue. Residue (40 g) were re-dissolved in MeOH-water (30:70) and partitioned exhaustively with dichloromethane (DCM, 3 L) in a separating funnel to yield aqueous (AQ) and DCM fractions. Fractions were similarly concentrated to dryness and respective yields recorded. Dried extract and fractions were refrigerated (4^oC) until needed.

***Phytochemical screening*:** Basic phytochemical screening was carried out on the crude methanol extract of the plant according to Sengar *et al*. [11] and the presence of secondary metabolites recorded.

***Drugs and reagents*:** Stilboesterol and oxytocin (Sigma-Aldrich, USA); methanol, NaCl, $NaClCO_3$, KCL, $CaCl_2$, $MgCl_2$,

$NaHCO_3$ and D-glucose (BDH Chemicals, England).

Preparation of physiological salt solution: The biological assay was carried out in Pharmacology laboratory, University of Benin, Edo State, Nigeria. Firstly, the physiological salt solution (PSS) was prepared according to Bafor *et al*. [12] by dissolving appropriate amounts of NaCl, $NaClCO_3$, D-glucose and KCL in distilled water in a calibrated beaker. $CaCl_2$ was dissolved in water separately to avoid cloudiness, and then added to the initial four-salt mixture to form the PSS with final composition in mM/L: NaCl 154.00, $NaHCO_3$ 5.95, D-glucose 2.78, KCl 5.63, and $CaCl_2 \cdot 2H_2O$ 2.05.

***Experimental animals*:** Healthy non-pregnant white albino rats (150–180 g) were utilized for this study. They were housed under standard conditions (27±5 °C and natural light and dark cycles) in the Central Animal House, University of Benin, and fed with standard animal pellets (Bendel Foods and Flower Meal, Edo State, Nigeria) and water *ad libitum.* Animals were handled in accordance with the Public Health Service policy on humane care and use of Laboratory Animals [13]. Ethical permission was obtained before the start of the experiments from the Animal Ethics Committee, Faculty of Pharmacy, University of Benin, Nigeria.

***Preparation of uterine tissue*:** Female rats previously primed with stilboesterol (1 mg/kg) for 24 h were used for the study as described by Bafor *et al* [12]. Animals were humanely sacriced if by cervical dislocation, uterine horns were immediately removed, placed in a petri dish containing previously warmed and aerated PSS. Connective and adhering tissues were removed from the isolated uterus and one horn was dissected in half to obtain a segment of the uterine horn of approximately 1–2 mm in length. Oestrogenized uterine segment obtained was mounted in a warmed tissue organ bath (10 mL) maintained at 37°C and containing aerated PSS. Organ bath was connected to an isometric force transducer (7003E- Ugo Basile, Varise, Italy) linked to a 17400 data capsule digital recorder with an inbuilt bridge amplifier (Ugo Basile, Varese, Italy). The tissue was equilibrated under resting tensions of 4.90 mN for 30–45 min or till regular contractions were obtained.

Determination of uterine contractility: MeOH extract (0.001–0.647 mg/mL) were added cumulatively to the organ bath containing isolated oestrogenized uterine tissue suspended in PSS to obtain concentration-response relationships according to Bafor *et al.* [12]. Concentrations used were previously determined in our laboratory constituting the total effect of the extract. Each concentration was allowed a contact time of 5 min before measuring the amplitude and frequency of contraction. Effect of extract and fractions on oxytocin-induced uterine contraction was similarly determined by adding cumulative concentrations of tested samples (0.001–0.647 mg/mL) to the tissue pre-contracted with 11.62 nM oxytocin. Furthermore, samples (10 mg/mL) were tested on isolated uterus pre-contracted with high KCL concentration (19.2 mM/mL).

***Determination of effect of extract in Ca^{2+}-free medium*:** In this experiment performed according to Bafor *et al* [12], PSS devoid of calcium was used, and ethylene diamine tetra-acetic acid (EDTA) was substituted. Oestrogenized uterine tissue was initially equilibrated for 30 min with former PSS and replaced with EDTA (1mM). Tissue was then equilibrated in this Ca^{2+}-free solution for 3–5 min (it was essential that contractions were not totally diminished during the experiment to allow for measurements). After

equilibration, oxytocin (6.7 µg/mL) was added and a contact time of 5 min was allowed. Without flushing, extract or fraction (10 mg/mL) was added. A contact time of 5 min was allowed for each sample before measuring amplitude and frequency of contraction.

RESULTS AND DISCUSSION

Extraction and fractionation of *P. guajava* stem bark yielded crude MeOH extract (15.01%), AQ fraction (35.95%) and DCM fraction (19.22%). Saponins, tannins, flavonoids, alkaloids, steroids, phenolic compounds and terpenoids were detected in the stem bark methanol extract of *P. guajava.* (Table 1). The involvement of flavonoids and phenols in uterotonic activity has been elucidated by Omodamiro *et al* [14].

Table 1: Phytochemical screening of *Psidium guajava* methanol extract

Phytometabolites	Inference
Cardiac glycosides	-
Tannnins	+
Flavonoids	+
Saponins	+
Alkaloids	+
Steroids	+
Phenolics	+
Terpenoids	+

+ = present, - = absent

All the three agents tested at 2-8 mg/mL except DCM fraction, displayed concentration-dependent increase in amplitude on non-pregnant rat uterus in the spontaneous contraction experiment (Panel A: Figures 1A-1C).

Although the extract showed greater force of contraction (1.109-1.177 g) than AQ fraction at lower concentrations, hence more active, both agents were equipotent (1.298 g) at the peak concentration(Panel B: Figure 2A) .

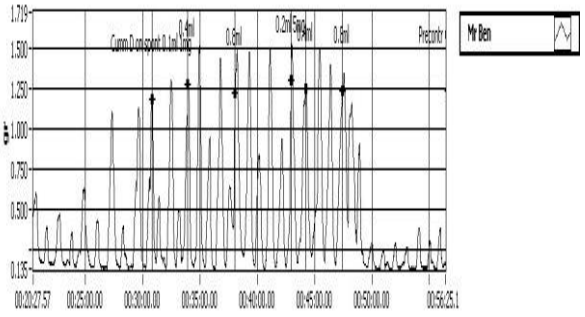

Figure 1A: Chart recording of *Psidium guajava* (PG) crude MeOH extract on uterine spontaneous contractions

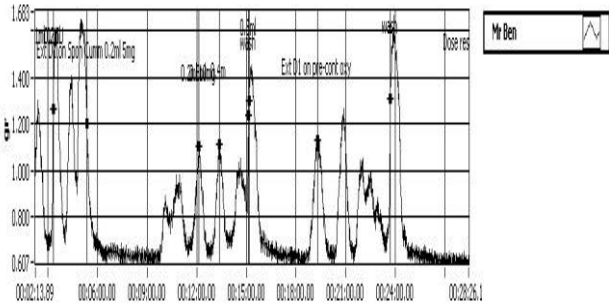

Figure 1B: Chart recording of aqueous (AQ) fraction of PG on spontaneous uterine contraction

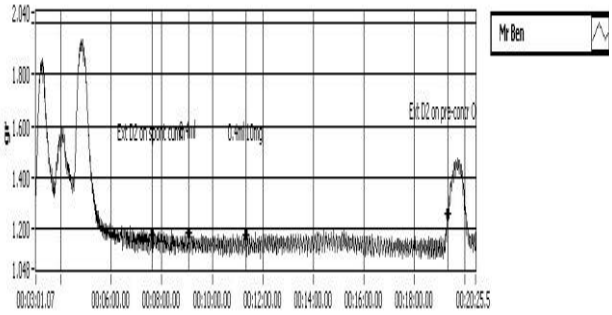

Figure 1C: Chart recording of DCM fraction of PG on spontaneous uterine contraction

PANEL A: Chart recordings of *Psidium guajava* in spontaneous uterine contractions in female rat

A similar trend was observed with contraction frequency in the case of extract (18-27 peaks/min) (Panel B: Figure 2B). However, the two fractions did not give concentration-dependent frequency response. Frequency increased by 75% at 4mg/ mL AQ fraction from initial value of 8 peaks/min, and then fell sharply to 25% at highest concentration. In considering both

parameters, crude MeOH extract was the most active uterine contractile agent (i.e. MeOH extract > AQ fraction > DCM fraction). This finding suggests oxytocic potential for extract, and also similar mechanism of action as the standard agonist, oxytocin. This result indicates ability of extract and AQ fraction to activate the pathways involved in stimulation of uterine contraction.

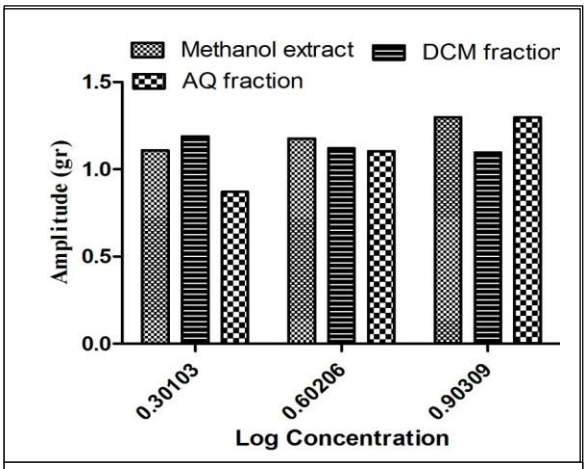

Figure 2A: Effect of *Psidium guajava* (PG) tested agents on amplitude of spontaneous uterine

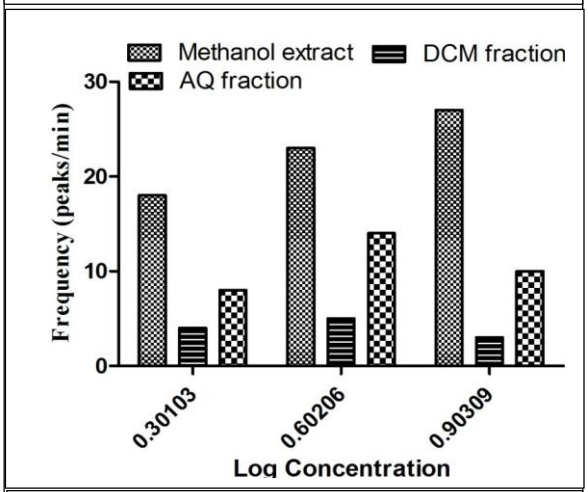

Figure 2B: Effect of PG on frequency of spontaneous uterine contraction

PANEL B: Effect of *Psidium guajava* on spontaneous uterine contraction in female rat

It is also in agreement with the reports of Watcho *et al*. [15] on *Ficus asperifolia* fruit, Ijeh *et al*. [16] on *Vernonia amygdalina* leaf, Bafor *et al*. [10] on *Ficus exasperata* leaf, Bafor *et al*. [12] on *Justica flava* leaf and Chanda *et al*. [17] on *Azanza garckeana* root.

Mechanisms for spontaneous contraction of uterine smooth muscle involving inhibition of K^+ channels and sarcoplasmic reticulum Ca^{2+} ATPase, and the non-estrogenic induction leading to increased Ca^{2+} entry through l -type Ca^{2+} channels and sarcoplasmic reticulum Ca^{2+} release have been documented [18]. The observed increased myometrial contractility by *P. guajava* extract and AQ fraction is possibly attributable to actions on post-ganglionic autonomic nerve endings with involvement of many receptors [19]. Moreover, Watcho *et al* [15] have adduced mechanism of oxytocic action of plant extracts to release of prostaglandins and contraction of the myometrial cells through more than one mechanism including the muscarinic, oxytocic and histamine receptors. The higher uterotonic potency of MeOH extract in this study, indicate the presence of polar bioactive oxytocic compounds. Apart from the isolation of pheophytin/pheophorbide derivatives and flavonoids from *Ficus exasperata* [10], bioactive oxytocic compounds is seldom reported for Nigerian plants. Elsewhere, Gruber and O'Brien [7] have reviewed active uterotonic compounds of plant origin on a global scale.

Apart from comparable contraction amplitude recorded with crude extract (1.472 g), AQ and DCM fractions tested at 1 mg/mL failed to potentiate 6.7 µg/mL oxytocin in pre-treated uterus suspended in normal PSS (Panel C: Figures 3A-3D). Furthermore, the extract was also active in enhancing contractile frequency response (42.85%) from reference value of 14 peaks/min (Panel C: Figure 3E). Since oxytocin normally binds

to its receptors to produce contractile effect [15,20], the potentiation of contraction frequency by *P. guajava* extract in this study might have proceeded via enhancement of the binding capacity of oxytocin to oxytocin receptors on myometrium tissues.

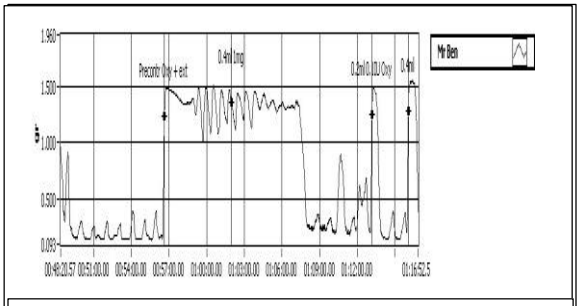

Figure 3A: Recording chart of crude extract of *Psidium guajava* (PG) on oxytocin pre-contracted

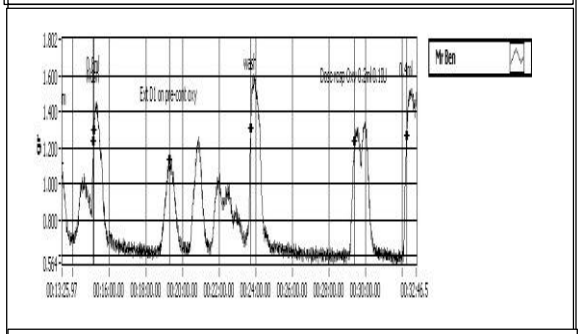

Figure 3B: Recording chart of AQ fraction of PG on oxytocin pre-contracted uterus

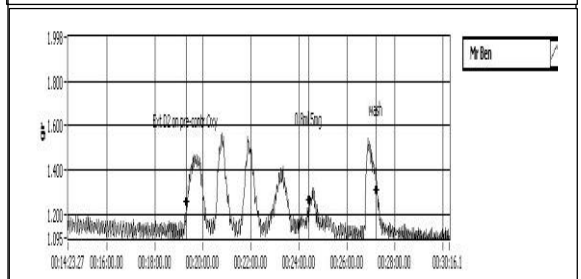

Figure 3C: Recording chart of DCM fraction of PG on oxytocin pre-contracted uterus

PANEL C: Effect of *Psidium guajava* on oxytocin pre-treated uterus on contractions in female rat

In the absence of Ca^{2+} from salt medium, all three tested agents (at 10 mg/mL) elicited comparable potentiation (15.40-15.41% increases in amplitude of contraction) of oxytocin (Panel D: Figure 4A-D).

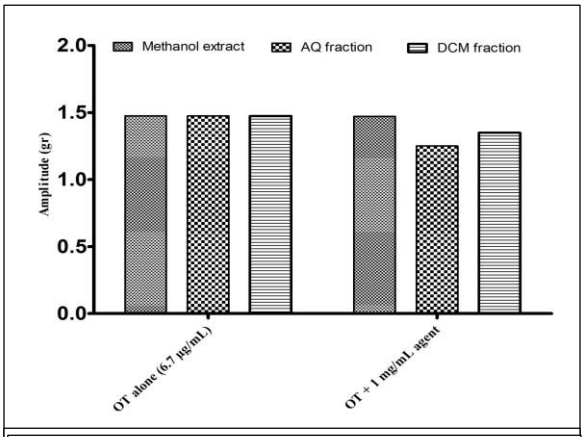

Figure 3D: Effect of PG tested agents on contraction amplitude in oxytocin pre-treated

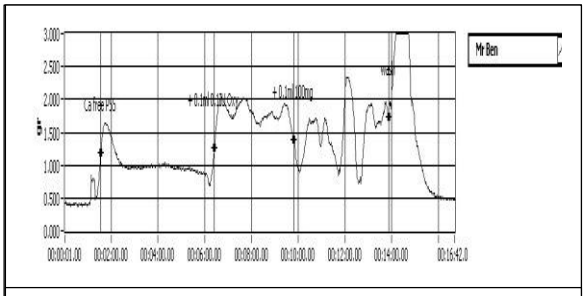

Figure 4A: Recording chart of crude extract of *Psidium guajava* (PG) on oxytocin pretreated uterus in calcium free medium

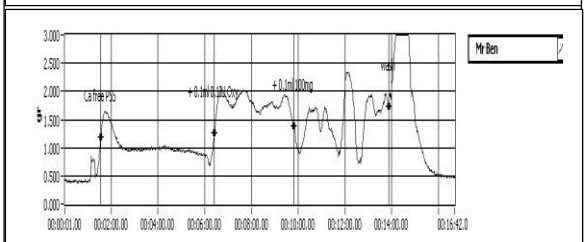

Figure 4B: Recording chart of AQ fraction of PG on oxytocin pretreated uterus in calcium free medium

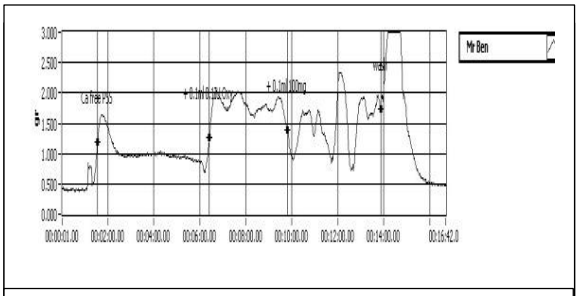

Figure 4C: Recording chart of DCM fraction of PG on oxytocin pretreated uterus in calcium free medium

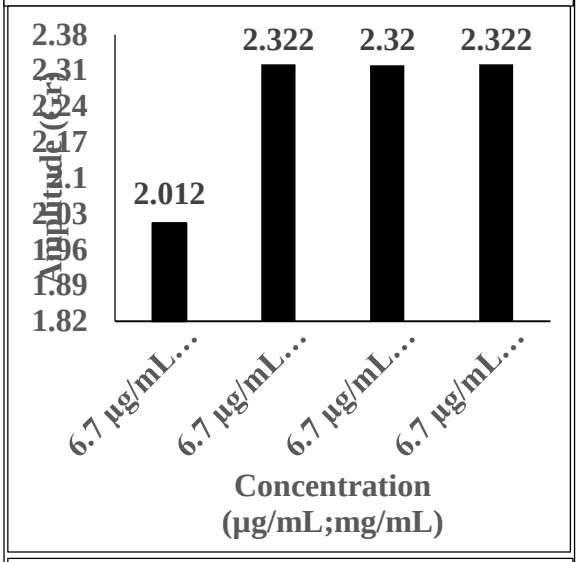

Figure 4D: Effect of tested agents of PG on amplitude changes in oxytocin pretreated uterus in calcium free medium

PANEL D: Effect of *Psidium guajava* on oxytocin pretreated uterus in calcium free medium

This finding is contrary to the frequently reported contraction inhibition in Ca^{2+}-free medium [12], and this portrays that the inclusion of the ions in PSS may be unnecessary for elicitation of uterine contractility. In another potentiation experiment, sustained potency of extract tested at 10 mg/ mL, also became noticeable in its ability to potentiaite (17.43%) high KCL concentration (80 mM) induced uterine contraction (Table 2, Panel E: Figures 5A-5D), which is consistent with the report of other workers in a different study [12]. It also possibly indicates *P. guajava* extract shares similar mechanism of action with KCL which Bafor *et al* [12] have linked to activation of L-type voltage-gated calcium channel leading to sustained tissue depolarization.

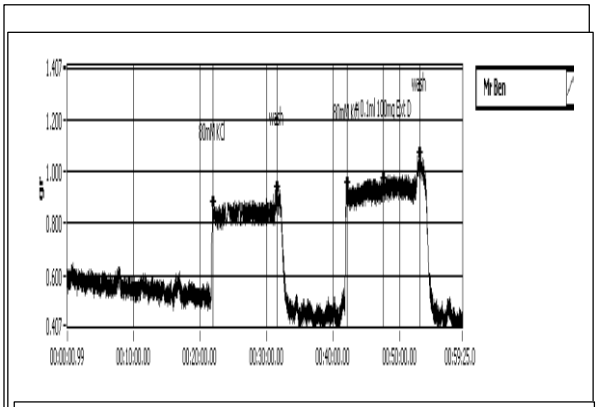

Figure 5A: Effect of *Psidium guajava* (PG) crude extract on high KCL-induced uterine contraction

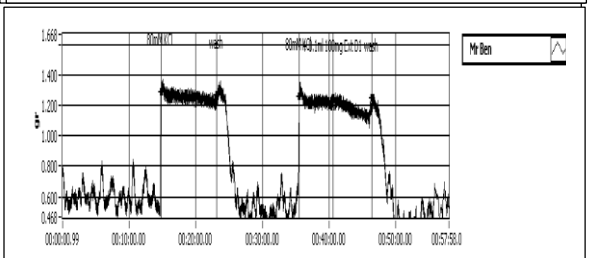

Figure 5B: Effect of *Psidium guajava* (PG) aqueous fracton on high KCL-induced uterine

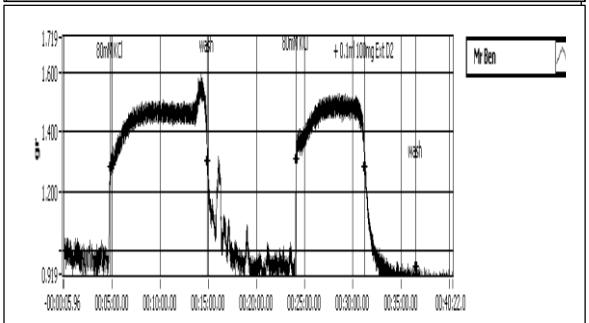

Figure 5C: Effect of *Psidium guajava* (PG) dichloromethane fracton on high KCL-induced uterine contraction

PANEL E: Effect of *Psidium guajava* on high KCL concentration-induced uterine contraction

Table 2. Effect of crude extract and fractions of *Psidium guajava* on high KCL concentration- induced uterine rat contractions

Concentration	Amplitude (gr)	% Change
80 mM KCL	1.509	-
KCL + DCM fraction (10 mg/ml)	1.360	- 9.87
KCL + AQ fraction (10 mg/ml)	1.532	+1.54
KCL + Crude MeOH extract (10 mg/ml)	1.772	+17.43

CONCLUSION

Psidium guajava stem bark induced spontaneous uterine contractions in non-pregnant female rat, also potentiated high KCL, and oxytocin in calcium-free medium. Both AQ and DCM fractions however inhibited contractions in oxytocin-pretreated rats using normal salt solution. MeOH extract was a more active oxytocic agent than the two fractions. Among the two fractions, AQ fraction was the more potent. This study has justified the traditional use of *P. guajava* stem bark in inducing labour.

Conflict of Interest: Authors declared that that there is no conflict of interest.

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