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Research Article

Contractile Effects of Methanol Extract of *Tacazzea apiculata* Stem on Albimo Rats Uterine Strips

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ABSTRACT

Abnormalities of uterine contractions during labour has been associated with many complications during childbirth including postpartum heamorrhage which may lead to maternal mortality. People use different medicinal plants to augment labour and to achieve a better outcome due to accessibility and affordability of such plants. Tacazzea apiculata stem has been in use for induction of labour at parturition in the Northern part of Nigeria but there is no scientific evidence backing up its use for this purpose. Hence, this study research the effect of the plant on uterus smooth muscle contractions in comparison with standard uterotonic agents such as oxytocin, prostaglandin, acetylcholine and potassium chloride in-vitro. The phytochemicals analysis was done using standard methods for such purpose. Cold methanol maceration was used for extraction. The LD50 was determined using Lorke's method. The uterus smooth muscle contractility activity of Tacazzea apiculata stem was determined by isolating the uterine strips of albino rats mounted in an organ bath connected to a power lab. Different concentrations of both the plant extract and standard uterotonics were administered. The methanol extract of Tacazzea apiculata stem contained alkaloids, flavonoids, saponins, tannins, steroids, terpenoids, and quinones. Acute toxicity studies revealed that the LD_{50} of the extract exceeded 5000 mg/kg, indicating a wide margin of safety. The extract elicited concentration-dependent contractions of the non-pregnant rat uterus. At concentrations ranging from 160 to 640 µg/ml, the extract demonstrated uterotonic activity comparable to standard agents, though effective at relatively higher doses. Statistically significant differences were observed between the response to the extract at 160 µg/ml and those of oxytocin (0.064 µg/ml), prostaglandin (6.4×10^{-4} µg/ml), acetylcholine (64×10^{-4} µg/ml), and potassium chloride (6.4×10^{-3} µg/ml). Additionally, a significant difference was noted between the extract at 160 µg/ml and 640 µg/ml. The methanol extract of Tacazzea apiculata stem exhibited significant uterotonic activity, likely attributable to its diverse phytochemical constituents,

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including alkaloids, flavonoids, and saponins.

INTRODUCTION

The uterus is a dynamic organ whose contractile activity is essential for menstruation, parturition, and postpartum uterine involution. Uterine contractility is tightly regulated by hormonal and neuroendocrine signals, including oxytocin and prostaglandins, which influence smooth muscle tone and responsiveness[1]. Aberrations in uterine contraction patterns are implicated in several reproductive health challenges such as dysmenorrhea, preterm labor, and postpartum hemorrhage conditions that significantly contribute to maternal morbidity and mortality, particularly in low-resource settings[2].In many African communities, traditional medicine plays a pivotal role in managing women's reproductive health, with plant-based remedies commonly employed to induce labor, regulate menstrual flow, or facilitate uterine evacuation[3]. *Tacazzea apiculata* (Oliv.), a member of the Apocynaceae family, is a climbing shrub indigenous to West and Central Africa. It has been traditionally used in parts of Nigeria for managing labor-related conditions, suggesting possible uterotonic properties. Preliminary phytochemical screening of *Tacazzea apiculata* has revealed the presence of bioactive constituents such as alkaloids, flavonoids, tannins, and saponins[4]. These compounds have been reported in other studies to exhibit pharmacological effects on smooth muscle tissue, including the uterus[5].

To date, controlled pharmacological studies evaluating its uterine contractility are lacking. This study was thus designed to assess the uterotonic effects of the methanol stem extract of Tacazzea apiculata on isolated uterine strips from albino rats, aiming to provide scientific validation for its traditional use and explore its potential as a safe, plant-based uterotonic agent.

METHODS

Collection and Identification of Plant Material

Fresh stems of *Tacazzea apiculata* Oliv. (Apocynaceae) were collected from Doka village, Bauchi Local Government, Bauchi state in June, 2021. The stems were dried until a constant weight were attained. The plant was identified by Malam Makama Hamza and authenticated at the Federal College of Forestry, Jericho, Ibadan, Oyo State by Dr. Samuel A. Odewo, a taxonomist. The specimen sample was kept in the college herbarium and assigned Voucher's number FHI-113439.

Year and Site of Experiment

The experiment was done in the department of Pharmacology and Toxicology, University of Jos, Jos, Nigeria in December, 2022 in the pharmacology laboratory.

Processing of the Plant Material

The stems of *Tacazzea apiculata* Oliv. (Apocynaceae) that has been dried to constant weight was triturated with mortar and pestle into powder. 50 g of the powdered *Tacazzea apiculata* stem was subjected to cold methanol maceration extraction by soaking with 500 ml of methanol with intermittent vigorous shaking every 30 minutes for 72 hours to facilitate extraction. The mixture was filtered with white muslin cloth. The filtrate was dried at room temperature until it was fully dried (constant weight). The dried extract was stored in a sample bottle and refrigerated, until it was ready for use.

Drugs and Chemicals

The drugs and chemicals used for the experiment includes Oxytocin (10 iu/ml, Labotocin, Laborate Pharmaceuticals, China), Prostaglandin (5 mg/ml,

Lutalyse injection, Zgetis, Zoetis Animal Health), Acetylcholine ($1x10^{-3}$), Estradiol (2 mg/ml Henbel Phango Pharmaceutical co. Ltd), Potassium chloride (15 g/10 ml) and methanol

Experimental Animals

Albino rats and Swiss albino mice were purchased from the Animal Experimental Unit, the Department of Pharmacology and Toxicology, University of Jos, Jos, Plateau state, Nigeria. They were housed under standard conditions (23°C±2^0C and 12 h light-dark cycle), and allowed free access to water and food. All experiments involving animals were performed according to internationally accepted guide for the care and use of laboratory animals, published by the US National Institutes of Health (NIH Publication No. 85, 23, Revised in 1985).

Ethical Approval

Ethical clearance was obtained from the ethical committee of the department of Pharmacology and Toxicology, University of Jos. The ethical clearance number was UJ/FPS/F17-0039.

Phytochemical Analysis

Phytochemical analysis was done on crude methanol extract using the standard procedures as described by Sofowara, Trease & Evans and Harborne [6,7,8]

Acute Toxicity Study

The method of Lorke was used to determine the LD_{50} (Lorke, 1983). The test was done in two phases.

Phase 1: Three groups of three mice each.

Group 1: 10 mg/kg of crude methanol extract of TA was administered to the three mice.

Group 11: 100 mg/kg of crude methanol extract of TA was administered to the three mice.

Group 111: 1000 mg/kg of crude methanol extract of TA was administered to the three mice.

Phase 2: Three groups of one mouse each.

Group 1, 11 and 111 were administered 1600, 2900 and 5000 mg/kg of TA methanol extract respectively.

The animals were observed for neurological disorders, respiratory distress, any behavioural changes, symptoms of toxicity and death.

Test for Oxytocic Activity of *Tacazzea apiculata* Extract on Isolated Uterus in

Comparison with some Uterotonic Agents.

The method of Wairimu[9] was modified. Estradiol (2 µg/ml) pre-treated non-pregnant female rats were humanely sacrificed by swift cervical dislocation. The uteri were promptly removed, cleaned of the connective tissues and cut into strips of about 2 cm in length. Each uterine strip was mounted in an organ bath of 25 ml capacity containing De Jalon solution The physiological salt solution was maintained at 37°C and continuously aerated with carbogen (5% carbon dioxide + 95% oxygen gas mixture). The upper end was attached to an isometric force transducer (ML500/A, AD Instrument) coupled to a Power Lab data acquisition system (Power Lab 8/30). Each preparation was subjected to a resting tension of 1.0 g and allowed to equilibrate for 30 min before it was challenged with the methanol extract of *Tacazzea apiculata*. The amplitudes of the contractions were measured in centimeter.

Comparision of Responses to the *Tacazzea apiculata* Extract with Uterotonic Agents.

The non-pregnant uterus that has been brought to oestrous by pre-treatment with 2 µg/ml estradiol was challenged with other standard drugs like oxytocin, potassium chloride, acetylcholine and prostaglandin, after an equilibration period of 30 min, normal myometrial contractions were recorded at baseline. Uterine contractile responses were elicited by adding oxytocin (0.064 µg/ml), acetylcholine (64 x 10^{-4} µg/ml), prostaglandin (6.4 x 10^{-4} µg/ml), Potassium chloride (6.4 x 10^{-2} µg/ml) and methanol extract of *Tacazzea apiculata* (160, 320 and 640 µg/ml) to the De Jalon's solution. Each dose of the drug and extract was allowed to act for 3 min and the amplitude of the contraction recorded by means of a force transducer and normal uterine smooth muscle contractions were recorded using an 8-channel recorder (Power Lab, model 8/30, AD Instruments, Australia). The amplitude of the crude extract and the standard drugs were compared by comparing their means using one – way ANOVA.

RESULTS

Phytochemical Analysis

Both qualitative and quantitative phytochemical analysis were carried out on methanol extract of TA. The following phytochemicals were detected: Alkaloids, saponins, tannins, flavonoids, steroids, terpenoids and quinonones. Alkaloids has the highest quantity while tannins was the least as shown in Table 1.

Acute Toxicity

The acute toxicity study showed no toxicity physically and neurologically in both phase 1 and phase 2 of the experiment. No mortality in the two phases of the experiment even at highest dose of 5000 mg/kg, thus the LD_{50} is greater than 5000mg/kg (Table 2).

Rhythmic Contraction of the Extract and the standard Drugs.

Figure 1-5 shows rhythmic contraction of the uterus on administering TA (40 µg/ml, 80 µg/ml, 160 µg/ml, and 320 µg/ml) extracts and standard drugs (oxytocin (0.004 µg/ml, 0.008 µg/ml, 0.016 µg/ml, and 0.032 µg/ml,), prostaglandin (1.6 x10^{-4} µg/ml, 3.2 x10^{-4} µg/ml, 6.4 x10^{-4} µg/ml and 1.28 x10^{-4} µg/ml), acetylcholine (4x10^{-4} µg/ml, 8 x10^{-4} µg/ml, 16 x10^{-4} µg/ml, and 32 x10^{-4} µg/ml) and potassium chloride (8 µg/ml, 16 µg/ml, 32 µg/ml and 64 µg/ml).

Comparison of Methanol Extract of *Tacazzea apiculata* (TA) with Oxytocin

Oxytocin (0.064 µg/ml) was compared with different concentrations (160 µg/ml, 320 µg/ml and 640 µg/ml) of methanol extract of TA. There was statistical significant difference between oxytocin concentration and 160 µg/ml of TA. P value = 0.023 (Table 3).

Comparison of Methanol Extract of *Tacazzea apiculata* (TA) with Prostaglandin

Prostaglandin (6.4 x 10^{-4} µg/ml) was compared with different concentrations (160 µg/ml, 320 µg/ml and 640 µg/ml) of methanol extract of TA. There was statistical significant difference between prostaglandin concentration and 160 µg/ml of TA. P value = 0.018 (Table 4).

Comparison of Methanol Extract of *Tacazzea apiculata* (TA) with Acetylcholine

Acetylcholine (64 x 10^{-4} µg/ml) was compared with different concentrations (160 µg/ml, 320 µg/ml and 640 µg/ml) of methanol extract of TA. There was statistical significant difference between acetylcholine concentration and 320 µg/ml of TA. P value = 0.044 (Table 5).

Comparison of Methanol Extract of *Tacazzea apiculata* (TA) with Potassium Chloride

Potassium chloride (6.4 x 10^{-3} µg/ml) was compared with different concentrations (160 µg/ml, 320 µg/ml and 640 µg/ml) of methanol extract of TA. There was statistical significant difference between potassium chloride concentration and TA concentrations of 160 µg/ml and 640 µg/ml. P values are 0.002 and 0.008 respectively (Table 6).

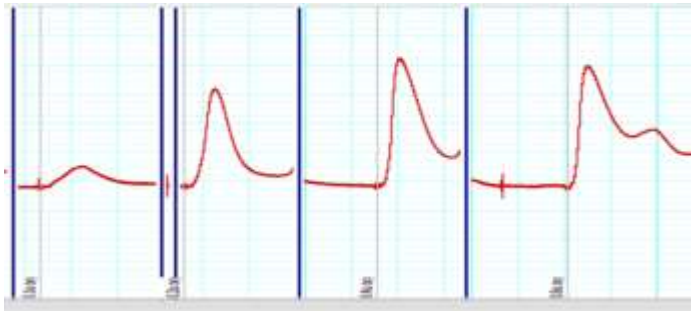
Table 1 : Phytochemical Properties of Methanol Extract of *Tacazzea Apiculata* (Ta) Stem

Phytochemical	Qualitative	Quantitative (w/w %)
Alkaloid	+	19.48
Flavonoids	+	0.12
Saponinins	+	1.04
Steroids	+	0.32
Tannins	+	0.03
Terpenoids	+	NQ
Quinones	+	NQ

Key + Means present NQ Means not quantified

Table 2 : Determination of LD_{50} of *Tacazzea Apiculata* by Lorke's Method

Phase	Dose Administered mg/kg	No of Animals used	Mortality
I	10	3	0/3
	100	3	0/3
	1000	3	0/3
11	1600	1	0/1
	2900	1	0/1
	5000	1	0/1

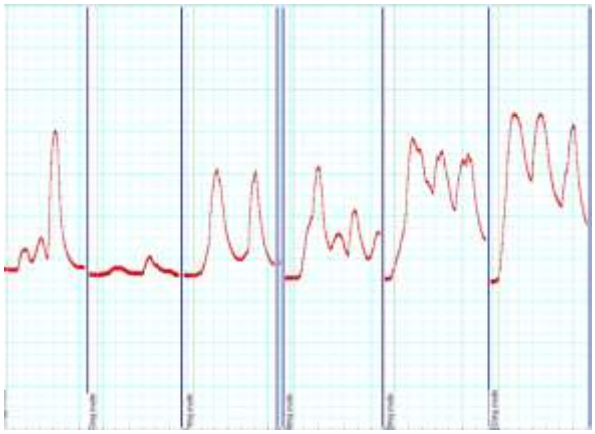


A B C D

Figure 1: Oxytocin (OT) (0.008 – 0.064) µg/ml Induced Uterine Smooth Muscle Contractions

Thick line – Washing; Thin line – Addition of Drug

A: OT 0.008 µg/ml B: OT 0.016 µg/ml
C: OT 0.032 µg/ml D: OT 0.064 µg/ml

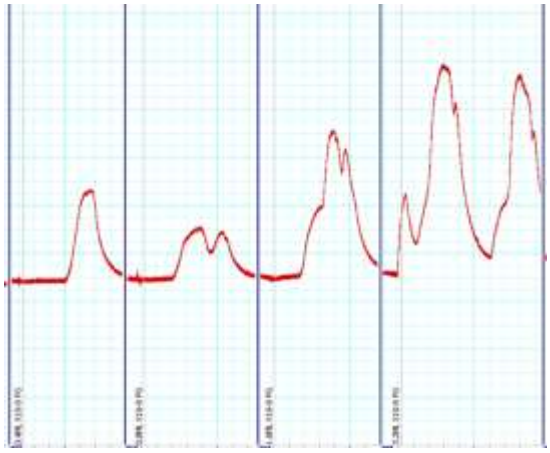


A B C D E F

Figure 2: *Tacazzea apiculata* Extract (40 – 1280) µg/mL Induced Uterine Smooth Muscle Contractions

Thick line – Washing; Thin line – Addition of Drug

A: TA 40 µg/ml B: TA 80 µg/ml C: TA 160 µg/ml D: TA 320 µg/ml E: TA 640 µg/ml F: TA 1280 µg/ml

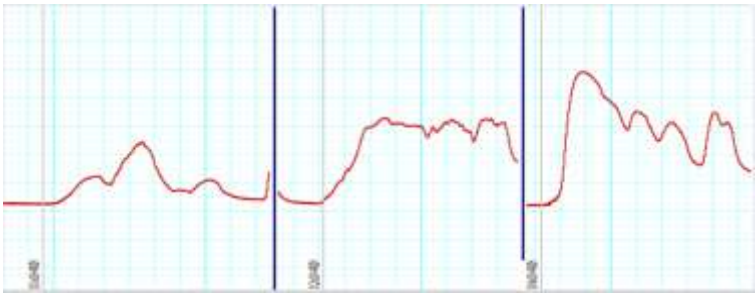


A B C D

Figure 3: Prostaglandin (PG) (1.6 x 10^{-7} - 1.28 x 10^{-7}) µg/ml Induced Uterine Smooth Muscle Contractions

Thick line – Washing; Thin line – Addition of Drug

A: PG 1.6 x 10^{-7} µg/ml B: PG 3.2 x 10^{-7} µg/ml C: PG 6.4 x 10^{-7} µg/ml D: PG 1.28 x 10^{-6} µg/ml

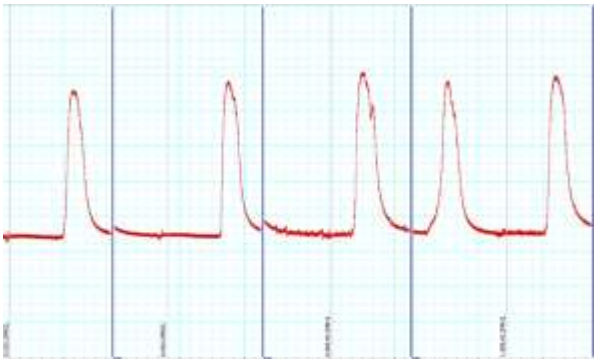


A B C

Figure 4: The Effect of Acetylcholine (Ach) (1.6 x 10^{-6}- 6.4 x 10^{-5}) mg/ml on Uterine Smooth Muscle Contractions

Thick line – Washing; Thin line – Addition of Drug

A: Ach 1.6 x 10^{-6} µg/ml B: Ach 3.2 x 10^{-6} µg/ml C: Ach 6.4 x µg/ml



A B C D

Figure 5: Effect of Potassium chloride (KCl) (8 x 10^{-3}- 2.56 x 10^{-1}) mg/ml Induced Uterine Smooth Muscle Contractions

Thick line – Washing; Thin line – Addition of Drug

A: KCl 8 x 10^{-3}mg/ml B: KCl 1.6 x 10^{-2}mg/ml

C: KCl 3.2 x 10^{-2}mg/ml D: KCl 6.4 x 10^{-2}mg/ml

Table 3: Comparison of Oxytocin with Crude Extract of *Tacazzea Apiculata*

Concentrations (µg/ml)	Uterine response (Amplitude in cm)
Rhythmic uterus contraction	3.43 ± 1.62
Oxytocin (0.064)	8.90 ± 0.15 $^{\Omega}$
Tacazzea apicculata (160)	6.63 ± 0.52 $^{*\Omega}$
Tacazzea apicculata (320)	8.63 ± 0.77 $^{\Omega}$
Tacazzea apicculata (640)	7.30 ± 0.47 $^{\Omega}$

Data are expressed as Mean ± SEM, n=5, Control versus other treatments, $^{\Omega}$ P < 0.05 and oxytocin versus extract *P<0.05

Table 4: Comparison of Prostaglandin with Crude Extract of *Tacazzea Apiculata*

Concentrations (µg/ml)	Uterine response (Amplitude in cm)
Rhythmic uterus contraction	3.43 ± 1.62
Prostaglandin (6.4 x 10^{-4})	9.00 ± 0.11 $^{\Omega}$
Tacazzea apicculata (160)	6.63 ± 0.52 $^{*\Omega}$
Tacazzea apicculata (320)	8.63 ± 0.77 $^{\Omega}$
Tacazzea apicculata (640)	7.30 ± 0.47 $^{\Omega}$

Data are expressed as Mean ± SEM, n=5, Control versus other treatments, $^{\Omega}$ P < 0.05 and prostaglandin versus extract *P<0.05

Table 5: Comparison of Acetylcholine with Crude Extract of *Tacazzea Apiculata*

Concentrations (µg/ml)	Uterine response (Amplitude in cm)
Rhythmic uterus contraction	3.43 ± 1.62
Acetylcholine (64 x 10^{-4})	9.26 ± 0.06 $^{\Omega}$
Tacazzea apicculata (160)	6.63 ± 0.52 $^{*\Omega}$
Tacazzea apicculata (320)	8.63 ± 0.77 $^{\Omega}$
Tacazzea apicculata (640)	7.30 ± 0.47 $^{\Omega}$

Data are expressed as Mean ± SEM, n=5, Control versus other treatments, **Ω** P < 0.05 and acetylcholine versus extract *P<0.05

Table 6: Comparison of Potassium Chloride with Crude Extract of *Tacazzea Apiculata*

Concentrations (µg/ml)	Uterine response (Amplitude in cm)
Rhythmic uterus contraction	3.43 ± 1.62
Potassium Chloride (6.4 x 10^{-3})	10.03 ± 0.05 $^{\Omega}$
Tacazzea apicculata (160)	6.63 ± 0.52 $^{*\Omega}$
Tacazzea apicculata (320)	8.63 ± 0.77 $^{\Omega}$
Tacazzea apicculata (640)	7.30 ± 0.47 $^{\Omega}$

Data are expressed as Mean ± SEM, n=5, Control versus other treatments, $^{\Omega}$ P < 0.05 and potassium chloride versus extract *P<0.05

DISCUSSION

Traditional birth attendants and pregnant women use herbs regularly for induction of labour. This practice suggests that the plants might be potent uterine stimulants, thus diligent scientific investigation of such plants is of paramount importance for discovering new oxytocic agents. The qualitative phytochemical analysis of the methanol extract of TA revealed the presence of the following phytochemical constituents: Flavonoids, alkaloids, tannins, saponins, cardiac glycosides, steroids, quinones and terpenoids. Components of TA phtochemicals have been shown to possess uterine stimulating effect [10, 11]. This finding was similar to the results obtained from the investigation of different plants with

uterotonic properties including *F. asperifolia, M. ciliatum*, *M. cecropiode* and *Nymphaea alba* all revealing the presence of saponins, tannins, flavonoids and steroids [10, 12, 13]. Also, the phytochemicals that are present in TA are present in aqueous extract of *Phytolacca dodecandra* (alkaloids, tannins, phenol, steroids and terpenoids) which also exhibited uterotonic effect and abortifacient properties [14]. The methanol extract of *Tacazzea apiculata* stem demonstrated significant uterotonic activity in non-pregnant rat uterine strips, supporting its ethnomedicinal use in the induction or augmentation of labour. The presence of phytochemicals such as alkaloids, flavonoids, saponins, tannins, terpenoids, and steroids might have contributed to its contractile effects, as these compounds are known to influence smooth muscle activity. Oestrogen activation is enhanced by flavonoids which promotes production of 'Connexin' a contractile protein. Connexin aids placenta removal by enhancing uterine contraction.[15] Oestrogen is a steroid; hence the presence of steroids might have contributed to the uterine contractile ability of methanol extract of TA.

The extract induced dose-dependent uterine contractions and exhibited a comparable pattern of activity to standard drugs including oxytocin, prostaglandin, acetylcholine, and potassium chloride, though at relatively higher concentrations. This suggests that the extract contains bioactive compounds capable of stimulating uterine smooth muscle through potentially similar or overlapping mechanisms. Although the extract required higher concentrations to achieve effects similar to conventional agents, its efficacy remains noteworthy, particularly in the context of traditional herbal medicine where crude extracts are commonly used. Previous studies have shown many plants possess uterine contractility activity similar to the plant under study. Such plants include *Euphorbia heterophylla* leaves[16], *Spondias mombin* [17], *Azanza garckeana* [18], *Launaea taraxacifolia* [19], *Uvariodendron kirkii* [9], *Steganotaenia Araliacea* [20], *Sida corybomsa* [21], *Nymphoea alba* [13], *and Monechema ciliatum* [12]. Furthermore, the high LD_{50} (>5000 mg/kg) indicates a wide margin of safety, reinforcing its potential therapeutic value.

However, the statistically significant differences observed between the extract and standard drugs highlight the need for further pharmacological characterization. Future studies should aim to isolate and identify the specific active constituents responsible for the observed effects and elucidate their mechanisms of action. Additionally, in vivo studies on pregnant models and toxicity profiling over longer durations would be essential to assess safety and efficacy in reproductive applications.

CONCLUSION

In conclusion, the methanol extract of *Tacazzea apiculata* stem possesses uterotonic properties that may validate its traditional use. The extract effectively induced uterine contractions in non-pregnant rats in a concentration-dependent manner and showed comparable activity to standard uterotonic agents, although at higher concentrations. These findings support the traditional use of *Tacazzea apiculata* in managing uterine conditions and warrant further investigation to isolate and characterize the active constituents responsible for its uterotonic effects. Its safety profile and biological activity make it a promising candidate for further investigation as a potential source of natural uterotonic agents.

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