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Acute toxicity, analgesic, and smooth muscle relaxant effects of the aqueous root extract of *Asparagus racemosus* from Kenya

Godfrey Mayoka[1], Peggoty Mutai[2]*, Faith Okalebo[2] and Daniel Juma[2]

[1]Department of Pharmacology and Pharmacognosy, School of Pharmacy, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya.
[2]Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi, KNH Campus, P. O. Box 19676-00202, Nairobi, Kenya.

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The plant *Asparagus racemosus* is used traditionally in Kenya for a variety of medicinal purposes including in the management of pain, asthma, and premature labour. However, a dearth of empirical studies means that there is limited information on the acute toxicity and pharmacological effects to support these uses by the local variety of the plant. The aim of this study was, therefore, to evaluate the acute toxicity, analgesic, and smooth muscle relaxation effects of the aqueous root extract of the plant. The powdered root of the locally sourced plant was extracted using decoction method. Analgesic activity was determined using both the hot plate and writhing methods whereas the effects on smooth muscle contraction were evaluated on the rabbit isolated ileum, rat uterus and guinea pig trachea. Acute toxicity was also evaluated in rodents. The LD_{50} of the root extract in mice was 505 mg/kg and dose-dependent analgesic effects were observed with 65% pain reduction at a dose of 400 mg/kg compared to 77% produced by morphine (20 mg/kg). Concentration-dependent smooth muscle relaxation effects were also observed on the isolated rabbit ileum and rat uterus. Similarly, guinea pig tracheal bronchodilation was observed to be concentration-dependent with over 90% relaxation at an organ bath concentration of 4 mg/ml. In conclusion, the aqueous root extracts of the Kenyan variety of *A. racemosus* produces analgesic, antispasmodic, tocolytic and bronchodilator activities. Future phytochemical analysis, isolation, and characterisation of candidate compounds responsible for these actions are recommended.

Key words: *Asparagus racemosus* extracts, analgesic, antispasmodic, tocolytic activities.

INTRODUCTION

Pain is one of the major manifestations of perturbed physiology and is the stimuli that prompts many people to seek medical attention. In turn, humanity has sought pain remedies from ancient times with nature playing a vital role in the quest for pain relievers (Jahromi et al., 2021).

Smooth muscle relaxation is a common approach in relieving abdominal pain and discomfort as well as preventing premature labor due to untimely uterine

*Corresponding author. E-mail: pckemei@gmail.com. Tel: +254 722 752 371.

contractions (Hafen et al., 2021). The search for analgesics and antispasmodics, in the context of traditional and alternative medicine, has led to the investigation of plants that may possess these activities. In conventional medicine, however, non-steroidal anti-inflammatory drugs, paracetamol (acetaminophen) and opiates (Thybo et al., 2019) are examples of popular pain remedies in the market while antispasmodics (Brenner and Lacy, 2021) and tocolytic agents (Haas et al., 2014) are exemplified by antimuscarinic agents and beta-two adrenoreceptor agonists, respectively.

Despite their usefulness, current analgesics and smooth muscle relaxants are beset by the limitations associated with undesirable side effects including drug addiction in the case of opioid-related analgesics. These agents are also contraindicated in various disease states (Machelska and Celik, 2018). These shortcomings inspire the search for safer and more affordable therapeutic interventions for the management of pain, abdominal discomfort due to intestinal hypermotility and premature labor caused by untimely uterine contractions.

Furthermore, to drive drug discovery and development programs, an area in which Africa continues to trail other continents, natural products are appealing starting points due to the rich biodiversity and the accompanying intellectual heritage that abounds among African communities (Thomford et al., 2020). Natural products remain a pivotal source of medicinal interventions for many communities worldwide including in Africa. Besides, nature-derived bioactive principles have served as important lead compounds in identifying potent semi-synthetic and synthetic drugs for numerous therapeutic indications (Newmann and Cragg, 2016).

The *Asparagus* genus, comprising over 300 species, represents a group of plants widely investigated for their nutritional, biological, antimicrobial, and pharmacological actions (Pegiou et al., 2020). Among the Asparagaceae family is the perennial dioecious plant *Asparagus racemosus*, a climber that grows in hardy, rocky environments and is native to the Indian subcontinent especially around the Himalayas. Growing from a tuberous rootstock, stems of *A. racemosus* can attain a height of above 5 m. The plant also inhabits other tropical and subtropical regions in Africa, China, Arabia, and Northern Australia (Singh and Geetanjali, 2016; Mfengwana and Mashele, 2019).

Previous phytochemical investigations of *A. racemosus* have led to the identification of steroidal saponins, alkaloids, flavonoids, and sterols as among its major constituents (Bhat et al., 2015). These phytochemicals bear diverse biological and pharmacological actions with steroidal saponins from *A. racemosus* reported to have, among others, anti-diabetic, antioxidant and hepatoprotective effects (Al Mamun et al., 2017). In addition, pharmaceutical preparations of the extracts of the plant have been studied for antiulcer effects as well as the ability to promote milk secretion among women reporting deficient milk production after delivery (Shaha and Bellankimath, 2017). Different isolated and characterized phytochemical compounds from the plant have been the subject of previous reviews (Zhang et al., 2018).

While considerable investigations have been conducted regarding the biological, pharmacological, and phytochemical profiles of the plant in other parts of the world, there is a dearth of information available in the literature about the species of the plant domiciled in Africa. Disparities in phytochemical contents and, therefore, biological, and pharmacological characteristics of plants, are known to occur along geographical and ecological lines (Atanasov et al., 2015). Empirical evaluation of the biological and pharmacological effects of the African variety of *A. racemosus* can provide new insights into the health benefits of this plant.

The plant *A. racemosus* is used among the Kalenjin community of Olenguruone in the Rift Valley region of Kenya to manage various types of pains, premature labor, and asthma, among other conditions. The aim of this study was, therefore, to use the knowledge of the folklore use of the plant as the impetus to investigate the potential analgesic, antispasmodic, tocolytic and bronchodilator activities of the extracts of the root of the local variety of the plant. We also conducted acute mice toxicity studies following the less studied intraperitoneal route of administration of the plant extract.

MATERIALS AND METHODS

Plant collection and preparation

The plant material was harvested in January 2019 from Olenguruone division, Kuresoi South constituency in Nakuru County and plant voucher specimens were deposited, after authentication, in the herbariums of University of Eldoret (WBMP/11/15/031) and the University of Nairobi (RI2019/08). The roots of the plant were washed using potable water and dried, chopped into small pieces and dried in a shade; after which they were milled into a coarse powder using a hammer mill (Muharata™). The powder was stored in an air-tight container, in a cool dry place away from light, pending further studies which were performed within six months of plant collection. Extraction was carried out by boiling 50 g of the powdered plant material in potable water for 15 min. The resultant decoction was evaporated to dryness overnight in an oven at 40°C, to give a brown residue.

Animal husbandry and ethical considerations

All experimental animals used were sourced from the Animal House of the Department of Pharmacology and Pharmacognosy, School of Pharmacy, University of Nairobi. These animals were housed in cages with free access to food and water and were fed on rat pellets obtained from Unga, Kenya Ltd. The animal housing was covered with sawdust and the animals kept under a 12-h light-dark cycle. Animal handling and all experiments were performed in accordance with requirements by the Institutional animal research committee and the standards and regulations of the European Union Animal Care and Experimentation Council (Guillén et al.,

2018).

Preparation of physiology solutions and drug standards

Morphine sulphate was obtained from Martindale Pharmaceuticals Ltd whereas diethylstilbestrol dipropionate and analytical grade glacial acetic acid were purchased from Glandorf (Germany) and Loba Chemie Pvt. Ltd, respectively. Normal saline was prepared by dissolving 9 g of analytical grade NaCI in 100 ml of distilled water. The physiological salt solutions; De Jalon solution comprising (mM): NaCl, 153.85; KCl, 5.64; $CaCl_2$, 0.55; $MgSO_4$, 0.08; NaOH, 12.5 and glucose, 2.78) (Watcho et al., 2011) and Tyrode solution consisting of (mM): NaCl, 137; KCl, 2.7; $CaCl_2$, 1.8; NaH_2PO_4, 0.4; $MgSO_4$, 0.25; $NaHCO_3$, 11.9 and glucose, 11.1 (Undale et al., 2012) were prepared as previously reported.

Acute toxicity test

Adult Swiss albino mice (21-27 g) were divided into four groups of five mice each. Each group of mice received a different dose of the *A. racemosus* root extract administered intraperitoneally. The doses were 250, 500, 750 and 1000 mg/kg, respectively. Intraperitoneal doses were prepared such that the final volume administered did not exceed 10 mg/kg for each animal (Chen et al., 2018). The animals were observed for 24 h for mortality or any untoward effects.

***In vivo* assays for analgesic activity**

Hot plate method

A hot plate was maintained at 55°C. Mice were placed in turns on the flat surface of the hot plate and the pain latency noticed by observing the time to pain reaction such as paw-licking, paw-raising, and an attempt to jump off the hot-plate (Hijazi et al., 2017).

Mice were initially screened for ability to respond to pain stimuli. Mice which did not respond within 30 s were excluded from the experiment. Consequently, twenty mice were divided into four groups of five mice each. Each group received, orally, either: *A. racemosus* aqueous root extract at a dose of 400 mg/kg, 200 mg/kg, normal saline (10 mg/kg) or morphine (10 mg/kg). Normal saline and morphine were used as the negative and positive controls, respectively.

A cut-off of pain latency of 30 s was applied throughout the experiment (that is, any mouse that did not display pain response within 30 s was removed from the hot plate until the next turn). The experiment for each dosing group was repeated after 10 min for 90 min. The percent pain inhibition was calculated according to the formula presented in Equation 1:

$$(\frac{Rt}{Rc} - 1)x100 \quad (1)$$

where Rt was the reaction time of mice to the test substance and Rc was the reaction time of mice to the negative control, normal saline.

Acetic acid writhing test

Pain was induced in mice using 0.2 ml of 1% v/v glacial acetic acid administered intraperitoneally. Morphine (20 mg/kg) and normal saline were used as positive and negative controls, respectively. Reactions to pain sensation characteristic of the classical writhing response (Gupta et al., 2015) such as licking of the site of injection, arching of the back, elongation of the body and extension of the hind limbs were recorded for a period of 30 min. The percent pain inhibition was determined using the formula presented in Equation 2:

$$\frac{W - Wt}{W} x100 \quad (2)$$

where W was the number of writhes observed in mice receiving the negative control substance (normal saline) and Wt was the number of writhes recorded in mice that received the test substance.

Effects on isolated rabbit ileum

A New Zealand White rabbit was sacrificed by cervical dislocation and the ileum harvested. The isolated ileum was set up at 37°C in a 20 ml organ bath containing Tyrode solution and aerated with a mixture of 95% CO_2 and 5% O_2 as described elsewhere (Pant et al., 2020). Contractions of the isolated ileum were recorded using an Ugo Basile® physiological recorder and peak height readings taken in mm using a meter rule. A 100 mg/ml stock solution of *A. racemosus* was prepared from which bath testing concentrations of 0.5, 1, 2 and 4 mg/ml were prepared. Duplicate testing was performed for each test concentration and the formula presented in Equation 3 used to calculate the percent reduction in the amplitude of the contractions of the ileum.

$$\frac{Baseline\ contractions - contractions\ with\ test\ substance}{Baseline\ contractions} x100 \quad (3)$$

Effects on the isolated rat uterus

One white albino virgin female rat weighing 184 g was pretreated with 0.1 mg/kg intramuscular injection of diethylstilbestrol. After 24 h of pretreatment, the rat was sacrificed by cervical dislocation and the gravid uterus removed and placed into a Petri dish containing De Jalon solution (Li et al., 2017). The two uterine horns were isolated and one of them set up at 37°C in a 20 ml organ bath containing De Jalon solution and aerated using 95% CO_2 and 5% O_2 as describe earlier (Agoreyo et al., 2017). Contractions of the isolated uterus were recorded using an Ugo Basile® physiological recorder and the peak heights readings taken using a meter rule.

Effects on isolated Guinea pig trachea

A Guinea pig was sacrificed by cervical dislocation, the trachea was isolated and placed into a Petri dish containing Tyrode solution. Subsequently, the trachea was cut transversely between segments and the eight segments were attached to each other back-to-back with a cotton thread before setting up in a 20 ml organ bath containing Tyrode solution at 37°C and aerated with a mixture of 95% CO_2 and 5% O_2 (Sadraei et al., 2019). Contractions of the isolated trachea were recorded using an Ugo Basile® physiological recorder and peaks recorded in mm using a meter rule. An *A. racemosus* stock solution of 100 mg/ml was prepared and five doses (10, 20, 40, 80 and 160 mg) were added to the bath giving final bath concentrations of 0.5, 1, 2, 4 and 8 mg/ml, respectively. Duplicate readings were taken for each test concentration.

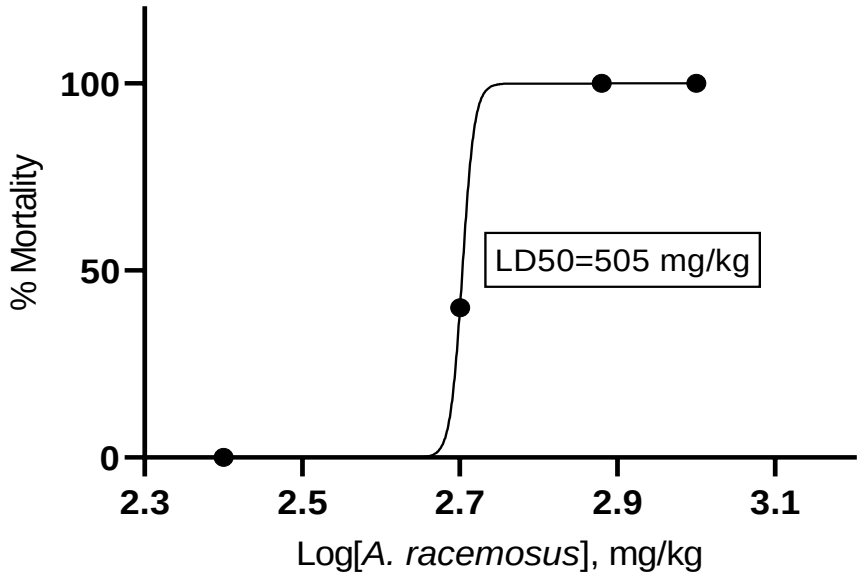

Figure 1. Acute toxicity of *A. racemosus* in Swiss albino mice. Graph generated using GraphPad Prism version 9.3.1 software. Five mice were used to test the safety of the water extract of the plant at each dose level.

Data analysis

The summary statistics were obtained by calculating the mean and either the standard deviation or the standard error of the mean. ANOVA was done to determine if there was a statistically significant difference in the responses across groups. The effective dose at 50% response was compiled by linear interpolation of the log dose response curves using GraphPad Prism version 9.3.1 software. The level of significance was set at 0.05.

RESULTS

Acute toxicity test

Twenty-four hours post-dose, none of the mice which received doses of 750 and 1000 mg/kg survived. As for the 500 mg/kg dose, two out of the five mice died. On the other hand, all the mice that received a dose of 250 mg/kg survived. *A. racemosus* root water extract was found to have an LD_{50} (a dose resulting in 50% mortality of mice) of approximately 505 mg/kg (Figure 1).

Analgesic screening test

As depicted in Figure 2, *A. racemosus* water extract displayed dose-dependent pain-suppressing activity as evaluated by the hot plate method.

Saline, used as a negative control, did not show any analgesic activity while morphine at 10 mg/kg displayed modest analgesic activity. The maximal pain suppression and time taken to achieve this effect are summarized in Table 1.

In the acetic acid writhing test, morphine (20 mg/kg) reduced pain sensation by 77%. On the other hand, *A. racemosus* root extract at 200 and 400 mg/kg displayed 43 and 65% inhibition in pain sensation, respectively (Figure 3).

Effects on the isolated rabbit ileum

A. racemosus root extract tested over a dose range of 0.5 to 4 mg/ml bath concentration, manifested a concentration-related reduction in the contraction of the isolated rabbit ileum. A concentration of 4 mg/ml led to over 90% relaxation of the rabbit ileum (Figure 4).

Activity on the isolated rat uterus and Guinea pig trachea

Tocolytic effects of *A. racemosus* root extract were evident when tested on the rat uterus. This effect was dose dependent at a dose range of 10 to 200 mg corresponding to 0.5 to 8 mg/ml bath concentrations. Linear interpolation of the concentration-response gave an EC_{50} value of 2.64 mg/ml (Figure 5a). On the other hand, the extract caused relaxation of the tracheal muscle as shown by the reduction in the intensity of peak heights from the Ugo Basile® physiological recorder. This change in peak heights was concentration-dependent with the highest tested concentration of 8 mg/ml causing maximum relaxation. The concentration resulting in 50%

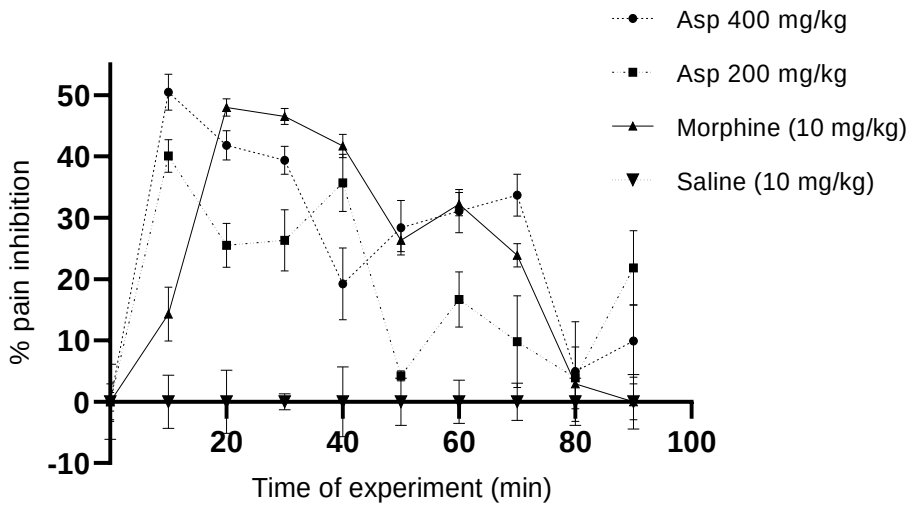

Figure 2. Pain inhibition of *A. racemosus* (Asp), saline and morphine (10 mg/kg) using the hot plate method. Graph generated using GraphPad Prism 9.3.1 and each line indicates the average response of five mice. Standard deviations are indicated by means of error bars.

Table 1. Pain suppression and time to maximal analgesic effects of the test substances.

Test substance	% Maximal pain suppression (SD)	Time to maximal effects (min)
A. racemosus (400 mg/kg)	50.5 (2.97)	10
A. racemosus (200 mg/kg)	40.1 (2.68)	10
Morphine (10 mg/kg)	48 (1.41)	20
Saline (10 mg/kg)	0	0

of tracheal relaxation was determined to be about 3.9 mg/ml (Figure 5b).

DISCUSSION

This study investigated the acute toxicity, antispasmodic, tocolytic and bronchodilator effects of the water extracts of the root of the plant *A. racemosus* obtained from the Rift Valley region of Kenya. Doses above 750 mg/kg were established to be toxic since all the mice that received these doses died within 24 h. We therefore concluded that a dose of 400 mg/kg and below was a safe range to work within this study because all mice that had been dosed with 250 mg/kg survived and only 2 of the 5 that were dosed at 500 mg/kg died within the same period. The LD_{50} of the extract, considering the intraperitoneal route, was determined to be about 505 mg/kg. In contrast to our results, a study by Ngeny et al. (2013) who used the aqueous root extracts of the plant and administered it to mice orally, did not show any lethal effects even at a dose of 5000 mg/kg. This disparity emphasizes the influence of the route of administration on toxicity profile. To the best of our knowledge, our work is the first to report the acute toxicity of the aqueous root extract of *A. racemosus* when administered intraperitoneally. This work, therefore, provides important preliminary acute toxicity data which is helpful to guide in the formulation of products from the plant intended for intravenous administration.

The failure by saline to induce analgesia, and the modest to high pain-relieving effects observed in mice that received morphine in both the hot plate and writing test methods, confirm that the observed analgesic activity of the *A. racemosus* aqueous root extract is unlikely to be a placebo effect. Furthermore, the dose-related increase in analgesic activity suggests a defined pharmacological action most likely mediated via established biochemical pathways that require further investigations. Interestingly, the peak analgesic activity of the *A. racemosus* root water extract occurred earlier (10 min) compared to that observed for morphine (20 min); possibly suggesting divergent analgesic mechanisms or the rapid absorption of the components of *A. racemosus*. Different chemical substances mediate early and late phase pain sensation (Abdulkhaleq et al., 2018). We believe that the aqueous

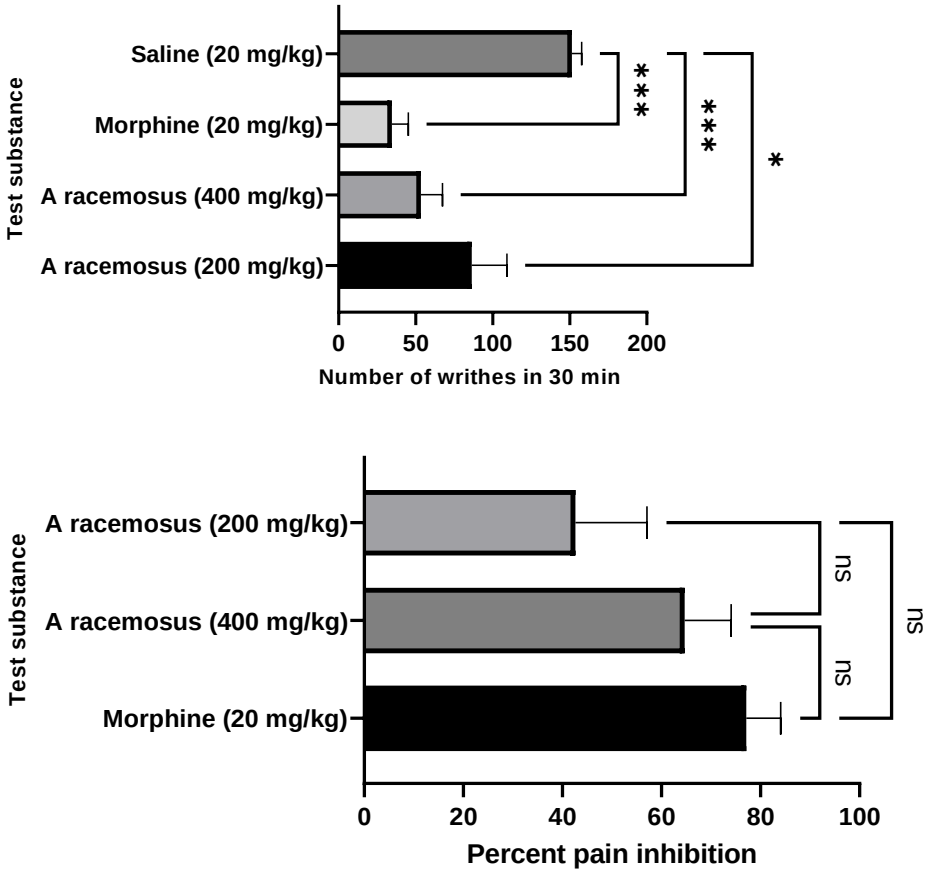

Figure 3. Pain inhibition of *A. racemosus* aqueous root extract, saline, and morphine: Top: average number of writhes in 30 min and, bottom: percent pain inhibition relative to the saline negative control. In both cases, each graph represents the average results of five mice. Error bars show the standard error of the mean and differences that are statistically significant are shown by an asterisk (* for P < 0.05; ** for P< 0.01; *** for p < 0.001) while ns means that the difference is not statistically significant.

extract of the plant contains bioactive substances that most likely modulate early phase pain sensation that is mediated via vasoactive amines including histamine- and serotonin.

In a previous study, the ethanolic extract of *A. racemosus* displayed 65% reduction in pain perception relative to the control vehicle-treated mice at a dose of 500 mg/kg (Karmakar et al., 2012). This approximates the pain inhibition observed in our study involving the aqueous extract where 400 mg/kg of the *A. racemosus* extract led to a similar magnitude of pain-relief. Since ethanol is a polar organic solvent, it can be assumed that the phytochemical principles that it is capable of extracting are similar, or closely related, to those extracted using water, the extracting solvent used in the present study. This rationale would explain the congruence in the observed analgesic effects of the two extracts.

The ileal relaxation effects produced by the *A. racemosus* root extract demonstrated a clear concentration-effect relationship. Similar trends were reflected in the inhibition of smooth muscle contractions of the rat uterus and Guinea pig trachea. Our results corroborate traditional uses of *A. racemosus* root extract as reported in Asia with regards to preventing threatened abortion, management of diarrhea besides other pharmacological functions that include anti-inflammatory and immunomodulatory. The versatile pharmacological actions of the plant have led to its elaborate use in Ayurvedic medicine for both infectious, nervous, and other disease conditions (Hasan et al., 2016). Among the chief phytochemicals with described pharmacological activities are flavonoids, saponins and sapogenins (Singh and Geetanjali, 2016; Thakur et al., 2018).

The antispasmodic activity observed in this experiment concurs with the reports by Dalvi et al. (1990). In their research, the powdered root of *A. racemosus* (2 g) resulted in reduced gastric emptying time comparable to that achieved by metoclopramide (10 mg), a synthetic dopamine antagonist. On their part, Venkatesan et al.

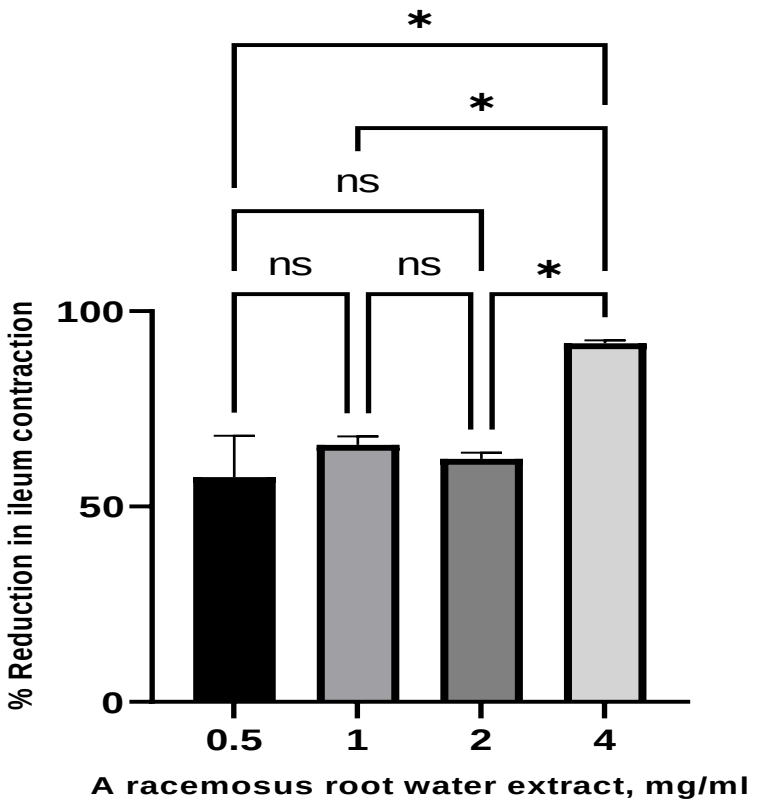

Figure 4. Effects of *A. racemosus* root water extract on isolated rabbit ileum. Graph generated using GraphPad Prism version 9.3.1 software. Each plotted point on the graph represents the average of two replicate readings. Standard deviations are indicated by error bars and differences that are statistically significant shown by an asterisk (P < 0.05). ns, differences not statistically significant.

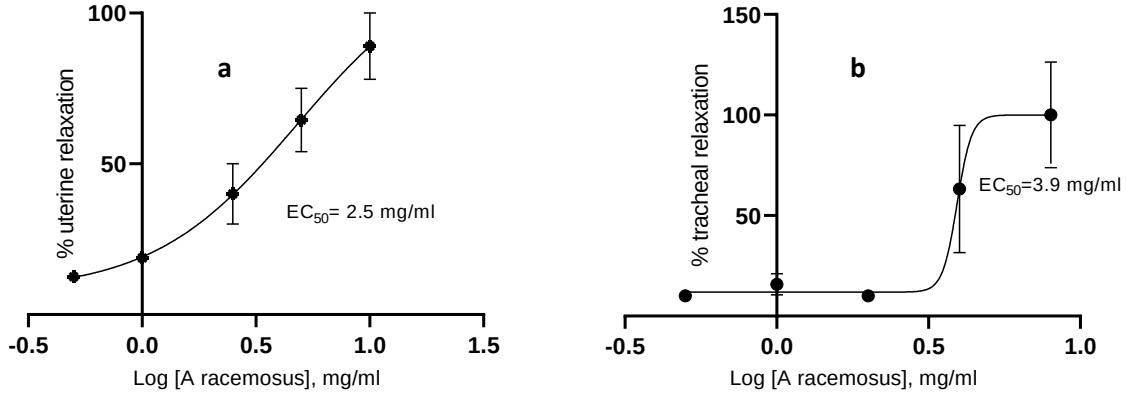

Figure 5. Effects of the aqueous *A. racemosus* root extract on: a) rat uterine and (b) Guinea pig tracheal contractions. Graphs generated using Graph Pad Prism version 9.3.1 software. Each plotted point on the graph represents the average of two readings. Error bars indicate the standard error of the mean for each reading.

(2005) observed that the ethanolic extract of the root of the plant prevented castor oil-induced diarrhea in mice at a dose of 200 mg/kg. Such an action has been related to the inhibition of the synthesis of prostaglandins involved in gastrointestinal contractions (Ruan et al., 2011). However, when ethyl acetate and acetone (less polar

solvents in comparison to water and ethanol) were used for extraction, acetylcholine-like gastrointestinal smooth muscle contractions were reported. These contractions could be antagonized by the cholinergic antagonist, atropine (Chawla et al., 2011). It is conceivable that the profile of bioactive phytochemicals with the two sets of solvent systems differ from a physicochemical and biological viewpoint, resulting in different pharmacological effects.

Our study also reports a spasmolytic effect in the isolated rabbit ileum in contrast to the report by Alok et al. (2013) but in agreement with the observations by Karmakar et al. (2012) who observed that polar organic and water extracts of *A. racemosus* showed anti-diarrheal activity in experimental animal models. This calls for studies by other investigators to reconcile this disparity which may arise from different factors including inherent differences in constituents of the plant based on geographical locations of the plant or experimental protocols.

Conclusion

The study shows that the aqueous root extract of the Kenyan variety of *A. racemosus* is safe for use intraperitoneally at a dose of up to 400 mg/kg in rodents. Furthermore, the extract portrays promising analgesic and smooth muscle relaxant effects, thereby providing empirical evidence for the folklore use of the plant in the Kenyan community. This study, therefore, contributes to bridging the gap on the medicinal value of the *A. racemosus* plant from East Africa which has been hitherto understudied. Further phytochemical analysis, isolation, and characterization of chemical constituents of this extract is recommended to enable a comprehensive profiling of the pharmacological actions of individual constituents of this plant, an important step in natural product-based drug discovery and development.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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