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EXPLORING THE ANTI-DIARRHEAL AND ANTI-HELICOBACTER PYLORI POTENTIAL OF ANDROSACE FOLIOSA: INSIGHTS FROM THE N-HEXANE LEAF FRACTION

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Abstract

Diarrhea is a common gastrointestinal condition marked by increased frequency and looseness of stools, representing a significant public health issue worldwide, especially among children under five. It contributes substantially to illness and death, with the highest impact seen in developing areas such as Sub-Saharan Africa and South Asia.

In this research, the aerial parts of Androsace foliosa were collected, properly identified, and processed to extract a hexane fraction. The spasmolytic activity of this extract was evaluated using isolated jejunal tissue from virgin female rabbits and anti-H. pylori was determined through disc diffusion method. Jejunal segments were suspended in an organ bath containing Tyrode's solution under controlled physiological conditions, and their spontaneous as well as potassium-induced contractions were measured. The hexane fraction was applied in varying concentrations to determine its dose-dependent relaxant effect, compared with verapamil, a known calcium channel blocker. Further investigation of calcium antagonism was performed by generating calcium concentration-response curves in a calcium-free, potassium-rich environment, with and without the plant extract. The results showed that the hexane fraction caused a significant, concentration-dependent relaxation of the rabbit jejunum, with EC50 values comparable to those of verapamil for both spontaneous and potassium-evoked contractions. Considering these promising spasmolytic and calcium channel blocking effects, further research is needed to isolate and identify the bioactive compounds responsible and to assess the in vivo efficacy and safety of A foliosa extracts in animal models. This study supports the potential of traditional medicinal plants as safe and effective options for antidiarrheal and anti-ulcer therapies.

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INTRODUCTION

Diarrhea is a gastrointestinal disorder, categorized by an increase in stool rate and change in consistency. Diarrhea is a gastrointestinal syndrome, characterized by a rise in stool frequency and altered uniformity (Balekar, 2014). Diarrhea is a common gastrointestinal disorder marked by the passage of three or more loose or watery stools within a day. It remains a significant global health concern, especially among children, where it contributes substantially to both morbidity and mortality. Diarrhea remains a major global health concern, particularly among young children, and is characterized by the passage of three or more loose or watery stools within a 24-hour period. [Afreen, G., & Ahirwar, D. 2023]. Clinically, it is classified into three main types based on duration and symptom severity: acute watery, acute bloody, and chronic. It is estimated that diarrhea leads to approximately 8 million deaths annually in children under the age of five. The burden is especially high in low-income regions, with sub-Saharan Africa and South Asia accounting for around 78% of these fatalities. Moreover, individuals aged five and older are affected by an estimated 2.8 billion episodes of diarrhea each year. Based on its duration and clinical symptoms, diarrhea is classified into three major types: acute watery diarrhea**,** acute bloody diarrhea, and persistent diarrhea**.** Annually, it causes close to 800,000 deaths among children under five**,** accounting for nearly 10% of all child fatalities**.** The highest impact is observed in developing regions**,** particularly in Sub-Saharan Africa and South Asia, which together account for about 78% of global child deaths due to diarrhea. Furthermore, the global incidence is staggering, with more than 2.8 billion diarrheal episodes reported each year among adolescents, adults, and older children. [Rawat, P et al. (2017). In third world countries, diarrhea is one of the main death triggering disease. This disease is the most concerning issue for under developing countries as millions of people die of diarrhea every year. Diarrhea is the second highest cause of death among children of age less than five years as they are more susceptible to this disease (Saralaya et al., 2010). To overcome this problem, the diarrhea disease control program has been initiated by World Health Organization (WHO) in which old treatment practices and prevention approaches are premeditated (Report, 2005). From ancient times the plants are considered as an important source for drug discovery and large number of different therapeutic substances are extracted from variety of plants. Medicinal plants have promising potential for the management of diarrhea (Dwivedi & Chauhan, 2012). Diarrheal disease is caused by a combination of factors. These include poor nutrient absorption, imbalance in gut bacteria, abnormal bowel movements, infections, metabolic issues, immune system problems, genetic conditions, and exposure to irritating chemicals. Certain substances like bacterial toxins, tumor-related hormones, bile acids, fatty acids, and inflammation-related chemicals can also trigger diarrhea. [Kotloff. K L et al., 2013]. Preventing and managing diarrhea involves several strategies such as promoting exclusive breastfeeding, using zinc supplements, practicing good hygiene, getting vaccinated, using oral rehydration solutions, and in some cases, taking antibiotics. [Boschi-Pinto et al., 2008] Although the number of deaths caused by diarrhea has dropped significantly since 2000, the disease still affects millions of people worldwide and remains a major health issue. Common antibiotics used to treat infectious diarrhea include ciprofloxacin, metronidazole, doxycycline, and amoxicillin, among others. However, frequent use of these medications can disrupt the natural balance of healthy bacteria in the gut, lower immune defenses, and cause allergic reactions. Over time, it can also lead to the development of antibiotic- resistant bacteria, making infections harder to treat. In some cases, antibiotic use may lead to the overgrowth of harmful bacteria like Clostridium difficile, which causes a specific type of diarrhea known as antibiotic-associated diarrhea. [Walker and Black, 2010]

Frontier in Medical & Health Research

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Fig # 1 Androsace foliosa

The diarrhoea causing organism commonly found in the affected sites of the patients such as Staphylococcus aureus, Escherichia coli, Campylobacter species, Clostridium difficile, Vibrio species, Salmonella enteritidis, Salmonella typhi and Shigella dysentriae were used for this study. The underlying mechanisms of diarrhea are multifactorial. Contributing factors include malabsorption of nutrients, disturbances in intestinal flora, abnormal gut motility, immune hypersensitivity, infectious pathogens, metabolic dysfunctions, chemical exposures, immune- related issues, hereditary traits, and hypersecretion induced by agents such as bacterial toxins, bile salts, fatty acids, endocrine secretions, and pro-inflammatory compounds. Current strategies to prevent and manage diarrhea focus on interventions such as exclusive breastfeeding, zinc supplementation, proper hygiene, vaccination, oral rehydration solutions (ORS), and antibiotics where appropriate. Despite achieving more than a 50% reduction in global diarrheal mortality between 2000 and 2013, the disease continues to pose a major public health burden due to its high incidence rate. These drugs usually have minor side effects and good potential activities against diarrhea. [D. Shah et al., 2012]

Loperamide, bismuth subsalicylate, and diphenoxylate are synthetic drugs frequently used to ease diarrhea symptoms. Loperamide helps manage diarrhea by slowing down bowel movements, allowing the intestines more time to absorb water from the stool, which results in firmer stools. Bismuth subsalicylate works through several mechanisms. It creates a protective layer on the intestinal lining to reduce fluid loss, has mild antibacterial properties, limits fluid secretion, and enhances the uptake of water and electrolytes by the intestinal cells. Diphenoxylate controls diarrhea by affecting the muscles in the intestinal wall, particularly the circular muscles, slowing down intestinal transit and giving the body more time to reabsorb fluids. [Johnson et al., 1986]. Treatment and avoidance of diarrheal diseases by using traditional medications are now encouraged by several worldwide organizations. Treatment and prevention of diarrheal diseases by using traditional prescriptions are now stimulated by numerous worldwide organizations. Plant kingdom contains huge pool of biologically important compounds with characteristic chemical properties that can prevent or cure diseases. Rheumatic fever, infant convulsions, and tooth ache can be cured by medicines. These medicines are also used to support digestion process, and also to dissolve internal blood clots. Some medicinal plants used in treatment of diarrhea are lemon, guava, mint, tea, turmeric (Joseph. Mini Priya, 2011).

Acute infectious diarrhea is often treated with antibiotics such as tetracycline, ciprofloxacin, norfloxacin, fleroxacin, cinoxacin, erythromycin, metronidazole, ampicillin, amoxicillin, doxycycline, vancomycin, and paromomycin. However, antibiotic therapy is frequently linked to undesirable side effects, including the reduction of beneficial gut and mucosal microbiota, suppression of the immune system, and

Frontier in Medical & Health Research

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the potential for allergic responses. Moreover, prolonged or inappropriate use of these drugs can contribute to the rise of antibiotic-resistant bacterial strains, making infections harder to treat. This disruption in gut flora can also trigger the excessive growth of harmful bacteria like Clostridium difficile, resulting in what is known as antibiotic-associated diarrhea. Due to the increasing resistance seen in many common infectious agents, there has been a renewed scientific focus on natural products as potential therapeutic alternatives. Medicinal plants have long played a role in traditional medicine systems and continue to serve as a foundation for drug discovery. In fact, many pharmaceutical drugs currently in use are either directly derived from plants or based on plant-derived compounds. The traditional use of herbal remedies to manage diarrhea and related gastrointestinal issues is well documented across various cultures. Medicinal plants are of immense importance to health of persons and communities. The medicinal activity of plants is due to presence of chemical substances that possess a specific physiological activity on human body. The most noteworthy bioactive constituents of plants responsible for pharmacological activities are alkaloids, tannins, flavonoids etc. [Pascaline J. et al 2011]

Material and Methods

2.1.1 Plant material

The plant Androsace foliosa was collected from Donga Gale, Ayyuba National Park Pakistan in July 2014. The plant was identified by a Botanist and the specimen is kept in herbarium of Botany Dept. G.P.G.C # 1. After washing carefully with tap water to remove dirt etc. the plant was shade dried. After drying plant was chopped and reduced to fine powder. Plant in the form of fine powder was kept in air tight container. Fresh plant was 5kg and after drying it weighed 2 Kg.

Preparation of fraction

Shade drying method was selected for drying of leaves of Androsace foliosa. After drying the leaves were pulverized with the help of electric grinder. After pulverization extraction was performed using the process of maceration. For extraction purpose leaves powder (500 grams) and soaked in n-hexane (1500 ml) for total of 7 days. The blend was filtered with the help of muslin cloth followed by using Whattman filter paper (No.1). The resultant filtrate was evaporated to dryness with the help of rotary evaporator beneath decreased pressure. After dryness the fraction provide a yield of 11 percent (w/w).

Animals

Virgin female rabbit weight (1-2 kg) was used for the study. The animals were reserved in well- ventilated room with standard housing circumstances with 12 hours dark/ light phase. Animal center organization of pharmacy and technology laboratory Abbottabad was the facility were animals were kept. They have excess to regular diet and un-limited excess to water. The animals were fasted 24 hours before the experiment. The research was performed with appropriate authorization by organization Animal Ethics board. Experiments were conducted according to the modern guiding principle for the concern of laboratory animals.

Ex-vivo Spasmolytic activity on rabbit jejunum

Rabbits (1.5-2.0 Kg) of either sex were used for the study. Animals were housed at standard animal conditions with 12 h light and dark cycle. Animals were maintained at 23-25 °C and were given standard diet and water ad libitum. Animals were provided with unlimited access to water and food but food was withdrawn 24 h prior to the experiment. The study was undertaken with due approval by Institution Animal Ethics Committee.

Isolated Tissue preparation

Rabbits were killed by cervical dislocation; abdomen was cut opened. Furthermore, jejunel portion was isolated out, and 2 cm long tissue strips were mounted in 10 mL tissue bath containing Tyrode's solution. The jejunel portion is aerated with mixture of Carbogen (5 % CO_2 and 95 % O_2) and maintained at 37°C. The composition of Tyrode's solution in mM was, (Nacl 136.9 g, Kcl 2.7 g, $Mgcl_2$ 1.1 g,$NaHCo_3$ 11.9 g, Glucose 5.6 g, NaH_2Po_4 0.4 g, and $Cacl_2$ 1.8g) pH maintained at (7.4). Tissue kept undisturbed for an equilibrium period of 30 minutes and preload tension of 1 g was applied. After stabilization, control response to a sub-maximal dose of acetylcholine (0.3mm) was obtained, and the tissue presumed stable

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only after the reproducibility of the said responses. Rabbit jejunum exhibits spontaneous rhythmic contractions when exposed under these experimental conditions, allowing the testing of spasmolytic (relaxant) activity directly without the use of an agonist (Taqvi et al., 2006).

Determination of calcium antagonistic activity

To assess whether spasmolytic activity of test substances was mediated through calcium channel blockade, a high concentration of K^+ (80mm) as Kcl was used to depolarize the preparations, and produce a sustained contraction. To obtain concentration-dependent inhibitory response standard drug and Afn-hexane leaves fraction were added in a cumulative fashion. The relaxation of intestinal preparations to standard drug and Af n-hexane leaves fraction pre-contracted with K^+, was expressed as percent of the control pre-contraction. To confirm the calcium antagonist activity of test substances, the tissue was allowed to stabilize in normal Tyrode's solution. After stabilization in order to remove Ca^{++} form the tissue the normal Tyrode's solution was replaced with Ca^{++} free Tyrode's solution containing EDTA (0.1mM) for 30 minutes. This solution was further replaced with Ca^{++}- free Tyrode's and K^+-rich having the following composition (mM) (Nacl 91.04 g, Kcl 50 g, NaHCo3 11.90 g, Mgcl2 1.05 g, NaH2Po4 0.42 g, EDTA 0.1Mm and Glucose 5.55 g). Control concentration-response curves (CRCs) of Cacl2 were obtained following an incubation period of 30 minutes. The tissue was pre-treated with Afn-hexane leaves fraction for 60 minutes to test the possible calcium channel blocking (CCB) effect when the control CRCs of Cacl2 were found superimposable. The CRCs of Cacl2 were reproduced in the presence of different concentration of standard drug and analyte.

Anti-Helicobacter pylori potential

The antibacterial efficacy of plant extract was assessed using the disc diffusion method. The experiment utilised Columbia Agar supplemented with 5% defibrinated sheep blood as the medium. Three Columbia agar plates were added with various isolates in saline phosphate buffer (10^8–10^9 cfu/ml), and discs impregnated with dried extracts were put to the plates and were incubated at 37 °C for 72 hours under microaerophilic conditions. The diameter of the inhibitory zone was evaluated after 72 hours.

Results

Ex-vivo anti-diarrheal activity on isolated rabbit jejunum

Effect of Af.n-hexane leaves fraction on Isolated Rabbit Jejunum in the presence of spontaneous and high K^+ (80 mM)-induced contraction.

The addition of the Af.n-hexane leaves fraction to the isolated rabbit jejunum preparations showed relaxant effect at the concentration range of 0.1-10 mg/mL. The Af.n-hexane leaves fraction produced dose dependent (0.01-10 mg) relaxant effect, with an EC50 value of 0.9 mM (0.3-1 mg/mL) when tested against spontaneous induced-contraction. When tested on high K^+(80 mM)- induced contraction Af.n-hexane leaves fraction showed EC50 value of 3.0mM (1-3 mg/mL) (Fig 1). The Af.n-hexane leaves fraction induced relaxant effect was found to be comparable to the effect of the standard drug verapamil which relaxed the spontaneous and K^+(80 mM)-induced contractions with EC50 value of 0.5 mM and 2.7 mM, respectively (Fig 2).

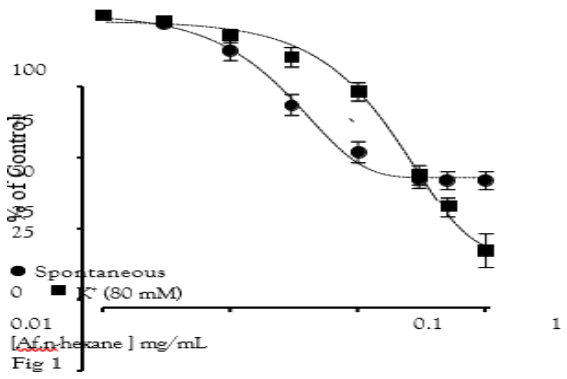


Fig 1

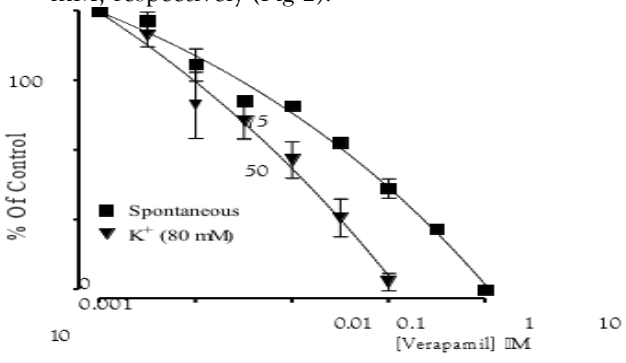


Fig 2

Volume 3, Issue 5, 2025

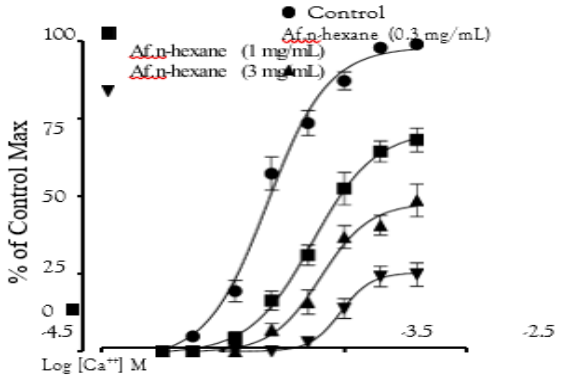
Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

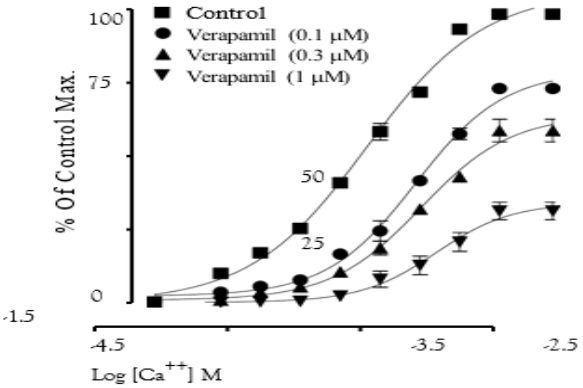
(Fig 1,2) Dose response curve showing the dose-dependent spasmolytic effect of Af.n-hexane leaves fraction and standard drug (verapamil) against spontaneous and high K^+ (80mM)- induced contraction on isolated rabbit jejunum (values are shown as mean ± S.E, n=5).

The effect of Af.n-hexane leaves fraction and standard drug verapamil induced- contraction on standard calcium curve on isolated rabbit jejunum.

To observe calcium blocking effect, the Af.n-hexane leaves fraction was also tested on the control concentration response curve of calcium, produced in the calcium free medium. In a set of experiments, the isolated jejunum preparations were pre-incubated with Af.n-hexane leaves fraction at a concentration range of 0.3-3 mg/mL for 35 minutes which shifted the concentration-response curves of Ca^{2+} towards right. Af.n-hexane leaves fraction inhibit the calcium curve approximately 40 %, 55 % and 75 % at the dose of 0.3 mg/mL, 1 mg/mL and 3 mg/mL respectively (Fig3). These effects were found to be similar to those produced by verapamil (0.1-0.5 µM) (Fig 4



(Fig 3)



(Fig 4)

(Fig 3, 4) Dose response curve showing the dose-dependent inhibitory effect of the Af.n-hexane leaves fraction and standard drug verapamil induced-contraction on calcium curve on isolated rabbit jejunum (values are shown as mean ± S.E, n=5).

Anti-Helicobacter pylori potential

After 72 hrs the petri dish was analyzed for zone of inhibition and was found that n-hexane fraction of Androsace foliosa extract showed 12.33 ± 0.72 mm zone of inhibition.

Discussion

Diarrhea is a gastrointestinal syndrome, characterized by a rise in stool frequency and altered uniformity (Balekar, 2014). In third world countries diarrhea is one of the main death triggering disease. Diarrhea is the second highest cause of death among children of age less than five years as they are more susceptible to this disease (Saralaya et al., 2010). To overcome this problem, the diarrhea disease control program has been initiated by World Health Organization (WHO) in which old treatment practices and prevention approaches are premeditated (Report, 2005). Medicinal plants have promising potential for the management of diarrhea (Dwivedi & Chauhan, 2012). These drugs commonly have minor side effects and good potential activities against diarrhea. Treatment and prevention of diarrheal diseases by using traditional prescriptions are now stimulated by numerous worldwide organizations. Some medicinal plants used in treatment of diarrhea are lemon, guava, mint, tea, turmeric (Joseph. Mini Priya, 2011).

In July 2014, the aerial parts of Androsace foliosa were collected from Donga Gali, located in Ayubia National Park, Pakistan. The plant was identified and confirmed by a botanist, and a voucher specimen was stored in the herbarium at the Botany Department of Government Postgraduate College No. 1. To preserve sensitive bioactive compounds, the plant material was gently washed to remove dirt and then dried naturally in the shade at room temperature. After drying, the plant was cut into smaller pieces and ground into a fine powder using an electric grinder. This powder was

Frontier in Medical & Health Research

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stored in airtight containers under suitable conditions. From the original 5 kilograms of fresh material, approximately 2 kilograms of dried powder was obtained.

For extraction, 500 grams of the dried powder was soaked in 1.5 liters of n-hexane at room temperature for seven days. The mixture was occasionally shaken to improve extraction. After this period, the liquid was filtered first through muslin cloth and then through Whatman No. 1 filter paper to remove solid residues. The clear filtrate was concentrated using a rotary evaporator under reduced pressure at temperatures below 40°C to avoid damaging heat-sensitive substances. The concentrated extract, referred to as the n-hexane fraction of Androsace foliosa (AF-Hex) was stored in a dark glass container. The extraction yield was about 11% of the dry plant weight.

Adult rabbits of both sexes weighing between 1 and 2 kilograms were used for the biological studies. They were kept in a well-ventilated room maintained at 23–25°C with a 12-hour light/dark cycle. Food and water were provided freely except for a 24-hour fasting period before the experiments, during which water was still accessible. All procedures involving the animals were approved by the Institutional Animal Ethics Committee and followed international ethical standards. For testing the spasmolytic activity, rabbits were euthanized humanely by cervical dislocation, and a section of the small intestine (jejunum) was removed. The intestine was cut into approximately 2 cm segments, which were placed in an organ bath filled with Tyrode's solution. This solution was continuously oxygenated with 95% oxygen and 5% carbon dioxide and kept at 37°C. Tyrode's solution contained essential ions such as sodium, potassium, calcium, and magnesium at physiological concentrations. A resting tension of 1 gram was applied to the tissues, and they were allowed to stabilize for 30 minutes. Tissue viability was confirmed by observing consistent contractions in response to acetylcholine (0.3 µM). Because rabbit jejunum naturally exhibits rhythmic contractions, it serves as an excellent model for evaluating the relaxing effects of substances without needing to stimulate contractions externally. To determine if the AF-Hex extract works by blocking calcium channels, muscle contractions were first induced using a high concentration of potassium (80 mM KCl). Potassium depolarizes the smooth muscle cell membrane, which opens voltage-dependent calcium channels and triggers contraction. After stable contractions developed, increasing concentrations of the AF-Hex extract were added to see if it could relax the tissue. Verapamil, a well-known calcium channel blocker, was used as a positive control. The degree of relaxation caused by both AF-Hex and verapamil was measured and compared.

To further investigate calcium channel blocking activity, the jejunum tissues were first stabilized in normal Tyrode's solution, then placed in a calcium-free Tyrode's solution containing EDTA to chelate any remaining calcium ions. After 30 minutes, the tissue was transferred to a high potassium, calcium-free Tyrode's solution to depolarize the muscle while preventing calcium influx. Following another 30 minutes of incubation, calcium chloride was added in gradually increasing amounts (ranging from 0.03 to 10 mM) to produce contractions and generate a calcium dose-response curve. Before constructing these curves, tissues were pretreated for 60 minutes with either AF-Hex or verapamil. A rightward shift in the calcium response curve, along with a decrease in maximum contraction, indicated that the extract was blocking calcium influx through voltage- dependent calcium channels, confirming its calcium channel blocking effect similar to that of verapamil. The addition of Af.n-hexane leaves fraction on the isolated rabbit jejunum was observed. The Af.n-hexane leaves fraction preparations showed a relaxant effect, as reflected by decrease in both amplitude and frequency of the spontaneous contractions on rabbit jejunum. The spontaneous movement of intestine is a phenomenon of alternative depolarization and repolarization and maximum depolarization is mediated through action potential produced via rapid influx of Ca^{2+} by means of voltage dependent Ca^{2+} channels. Enhanced contraction of smooth muscle is due to increase availability of Ca^{2+} in cytoplasm is the preparation through activation of several neuro- endocrinal agonist i.e acetylcholine and serotonin The addition of the Af.n-hexane leaves fraction to the isolated rabbit jejunum preparations showed relaxant effect at the concentration range of 0.1- 10 mg/mL.The Af.n-hexane leaves fraction produced dose dependent (0.01-10 mg) relaxant effect, with an EC50value of 0.9mM (0.3-1 mg/mL),when tested against

Frontier in Medical & Health Research

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spontaneous induced-contraction. When tested on high K^+ (80 mM)-induced contraction Af.n-hexane leaves fraction showed EC50 value of 3.0 mM (1-3 mg/mL), (Fig 3.22 a). The Af.n-hexane fraction induced relaxant effect was found to be comparable to the effect of the standard drug (verapamil)

This study explored the effects of the n-hexane extract derived from the leaves of Androsace foliosa on the smooth muscle of isolated rabbit jejunum. The jejunum exhibits natural rhythmic contractions driven by electrical impulses, which are primarily regulated by calcium ions entering muscle cells through specific channels, resulting in muscle contraction. When varying concentrations of the extract (0.1 to 10 mg/mL) were applied, it caused a progressive relaxation of these spontaneous contractions. Both the strength and frequency of the muscle contractions decreased in a dose-dependent manner. The concentration at which the extract reduced contractions by 50% was approximately 0.9 mg/mL, indicating a moderate muscle-relaxing effect without completely inhibiting muscle activity.

Additional experiments investigated the extract's ability to counteract contractions induced by high potassium levels (80 mM). High potassium concentration depolarizes the muscle cell membrane, leading to the opening of calcium channels and a significant influx of calcium ions that cause strong muscle contractions. The extract was able to relax these potassium-induced contractions, although at higher concentrations, with an effective dose (EC50) close to 3 mg/mL. This suggests that the extract may interfere with calcium channel activity, reducing calcium entry into the muscle cells. To support this hypothesis, the extract's effects were compared with verapamil, a known calcium channel blocker and muscle relaxant. Verapamil exhibited similar results, with an EC50 of 0.5 mg/mL for spontaneous contractions and 2.7 mg/mL for potassium-induced contractions. The similarity between the effects of the extract and verapamil strengthens the notion that the extract acts by inhibiting calcium influx into muscle cells.

Further tests conducted in a calcium-free environment, where muscle contractions were triggered by gradually increasing calcium levels, showed that pretreatment with the extract (at doses of 0.3, 1, and 3 mg/mL) shifted the calcium response curve to the right and reduced the maximum contraction strength. The extract inhibited calcium-induced contractions by roughly 40%, 55%, and 75% at these respective concentrations. These findings were consistent with verapamil's action, confirming that the extract reduces contractions by blocking calcium entry. Taken together, these results suggest that the n-hexane extract from Androsace foliosa leaves can modulate intestinal smooth muscle activity by relaxing excessive contractions. This characteristic may have therapeutic potential in managing disorders like diarrhea and intestinal spasms, which involve hyperactive muscle contractions. By limiting calcium influx into the muscle cells, the extract helps reduce contractions in a manner similar to established muscle relaxants. In conclusion, the n-hexane fraction of Androsace foliosa leaves exhibits notable muscle-relaxing properties through the blockade of calcium channels. Future studies should focus on identifying the active compounds responsible and assessing their effects in whole organisms to fully evaluate their medicinal potential. The intermediate potential of Androsace foliosa against H. pylori was showed and provided the evident of its efficacy against gastric ulcer.

CONCLUSION

Diarrhea is a common gastrointestinal disorder marked by frequent loose or watery stools, posing a major global health challenge, especially for children under five, causing nearly 800,000 deaths annually. It is most prevalent in developing regions like Sub-Saharan Africa and South Asia. Causes include infections, impaired absorption, gut flora disruption, and toxins. Standard management involves hydration, zinc supplementation, vaccination, sanitation, and antibiotics, though antibiotic overuse can disrupt gut microbiota and lead to resistance. Synthetic anti-diarrheal drugs like loperamide, bismuth subsalicylate, and diphenoxylate reduce intestinal motility and fluid secretion. Increasingly, traditional medicinal plants are being explored for diarrhea treatment due to their bioactive compounds, though more scientific validation is needed. In this study, n-hexane extracts of Androsace foliosa aerial parts from Pakistan were tested on isolated rabbit jejunal tissues. The extract showed dose-dependent relaxation of intestinal contractions, similar to the calcium channel blocker

Frontier in Medical & Health Research

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verapamil, indicating spasmolytic activity via calcium channel blockade. Phytochemical screening revealed alkaloids, saponins, flavonoids, triterpenes, tannins, and coumarins in the extracts. These findings support the traditional use of A. foliosa in diarrhea management and highlight its potential as a plant-based therapeutic. Further isolation and characterization of its active compounds are recommended to develop effective treatments.

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