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Orignal Article

PHYTOCHEMICAL SCREENING, FREE RADICAL SCAVENGING ACTIVITY, INVITRO ANTIOXIDANT AND ANTIHELMINTHIC ACTIVITY OF *MUCUNA PRURIENS* SEED

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Abstract: ***Objective: Mucuna pruriens (M. pruriens) is a legume plant that has been used in traditional medicine for its various therapeutic benefits. It is one of the essential ingredients in herbal medication used to treat a variety of illnesses, including male sexual dysfunction. In this study phytochemical capabilities, In-vitro antioxidant capacity, and antihelminthic activity of methanolic extract of M. pruriens seeds were investigated.The preliminary phytochemical screening of M. pruriens was carried to find out the active constituents responsible for the pharmacological activities of the seed extract.The antioxidant activity of the M. pruriens were performed by using the (2,2 -Diphenyl -1- picrylhydrazyl) DPPH radical scavenging assay and Nitric oxide radical scavenging assay at different concentration compared with standard Vitamin C. Also, the seed extract was tested for its antihlementhic activity against two intestinal nematodes, Ascaridia galli(A. galli) and Cestode parasites Raillientina tetragona (R. tetragona).The present study supports that M. pruriens contains high level of antioxidant such as phenols, flavonoids and alkaloids which have been found to scavenge free radical and reduce oxidative stress in body. M. pruriens seed extract exhibits 50% more inhibition activity at the concentration of 330µg/ml. The results also shows that M. pruriens seed extract that contains alkaloids and tannins, which have been shown to possess antihlementhic activity against intestinal worms and parasites.In conclusion, methanolic extract of M. pruriens exhibits antioxidant and antihelminthic property which could be used to develop new drugs for the treatment of oxidative stress-related diseases and parasitic infections on humans.***

Keywords: Antihelminthic , *Mucuna pruriens* seed.

Mrs. Chitra Kalyanaraman. She completed Ph.D. in Pharmacology and Environmental Toxicology, University of Madras, Taramani, Chennai. She achieved an exceptional first-class distinction. Furthermore, she attained a remarkable first-class distinction in her M. Phil program in Environmental Toxicology at the University of Madras, Chennai. Additionally, she achieved a first-class honor in her Master of Science program in Environmental Science at Anna University, Chennai.

Dedication: I would like to express my heartfelt gratitude and admiration for Dr. P.D. Gupta's unwavering commitment and outstanding achievements in science and research, which have touched the lives of so many people. As we celebrate his 85th birthday, it brings me tremendous pleasure to dedicate my research endeavors to him

INTRODUCTION

Nature has provided a comprehensive reservoir of remedies for alleviating human ailments. In India, there is an abundance of approximately 8000 species of medicinal plants, which have been recognized as the oldest form of healthcare known to humanity. Numerous medicinal plants that have existed for thousands of years play a vital role as a source of innovative bioactive compounds. Different parts of plants, including stems, barks, leaves, fruits, and seeds, possess distinct physiological effects on the human body. In most industrialized nations, natural products are readily accessible, allowing for the exploration of their biological and chemical characteristics, which holds significant importance. The appreciation and exploration of the extensive diversity of plants accessible to early humans played pivotal roles in the advancement of modern civilization.

Free radicals and antioxidants play a dual role in the body, as they can be either beneficial or harmful. When the body cannot gradually remove an excess of free radicals, a situation known as oxidative stress occurs. This phenomenon has gained increasing attention in recent years, particularly in relation to its impact on testicular dysfunction in experimental animals and infertility in humans [1,2]. Environmental stress is recognized as one of the contributing factors to the generation of free radicals, particularly Reactive Oxygen Species (ROS), which can cause damage to biomolecular structures. Consequently, this alteration in functional characteristics can result in cellular dysfunction and even cell death [3]. The accumulation of Reactive Oxygen Species (ROS) has a cumulative impact on various organ systems, leading to oxidative stress, which plays a significant role in the development of cancer, cardiovascular diseases, as well as age-related and neurodegenerative disorders like Parkinson's disease [4,5]. In living systems, the regulation of cellular ROS is governed by a complex antioxidant machinery [6]. Seminal plasma contains significant ROS, such as superoxide anion, hydroxyl radical, and hydrogen peroxide, which can affect cellular function. However, the presence of antioxidants in seminal plasma can counteract these effects [7].

Plants are frequently abundant, cost-effective, and contain numerous phytochemical compounds. This has led to their utilization in traditional and alternative medicine, as well as in research focused on health-promoting compounds. Understanding the phytochemical composition and biological activity of bioactive extracts is critical. Consequently, it is vital to investigate the toxicity and potential side effects associated with plant extracts [8]. Phytochemicals are non-nutritive plant chemicals that possess protective or preventive properties against various diseases. Secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants contribute to their therapeutic efficacy [9]. Although non-essential for human vital functions, these chemicals are produced by plants to safeguard themselves. Recent research indicates that they also offer protection to humans and animals against diseases and pathogenic microorganisms.

According to Kyne et al. [10], helminthiasis is considered the leading infectious disease, causing significant morbidity and mortality in both humans and livestock animals. The World Health Organization [11] estimates that nematode infections are anticipated to affect more than one-fifth of the global human population, resulting in approximately 1.5 billion cases. Bundy [12]) explains that helminth infections predominantly occur in tropical regions and pose substantial health risks, contributing to the prevalence of conditions such as malnourishment, anemia, eosinophilia, and pneumonia. Perry and Randolph [13] suggest that the impact of parasitic diseases in the developing world is primarily indirect, leading to potential productivity losses. Additionally, Monteiro et al. [14] highlight the significance of gastrointestinal helminth infections in grazing livestock, as they are among the most common and economically important diseases affecting these animals.

Antihelminthic, also known as worm medicines, are drugs used to eliminate and eradicate parasitic worms in both human and animal bodies by killing or eliminating them. Although deworming drugs are commonly used, there are concerns about their potential side effects. Therefore, it is important to explore alternative treatments that are affordable, potent, and free from side effects. One potential alternative is the use of traditional or natural plants [15,16]. Hence, the present study aims to assess the antihelminthic activity of the methanolic extract obtained from the seeds of *M. pruriens*.

M. pruriens, a widely used drug in alternative medicine, has gained popularity for its therapeutic properties. The seeds of *M. pruriens* have shown

effectiveness in treating neurological disorders [17] and male infertility [18]. Previous studies have reported on various medicinal properties of *M. pruriens* seeds, including antidiabetic, anti-inflammatory, antipyretic, antiparkinson, and aphrodisiac properties [19,20]. The potential efficacy of *M. pruriens* seeds in degenerative disorders has sparked further research into its multifaceted pharmacotherapeutic applications. Building upon these findings, the present study aims to identify the phytochemical constituents and evaluate the antioxidant and antihelminthic activities of the seed extract.

MATERIALS AND METHODS

Collection and Preparation of the Seed Extract: The Mucuna pruriens seeds were procured from a rural pharmacy in Chennai, Tamil Nadu, India. These seeds underwent certification by the principal botanist affiliated with the Plant Anatomy Research Centre (PARC) in Chennai. Subsequently, the seeds were subjected to shade drying and coarsely powdered. A measured amount of the powder was soaked in 100% methanol and left at room temperature (22± °C) for 96 hours. This process was repeated thrice after filtration. The resulting extract was concentrated using a water bath to yield a semi-solid, viscous brown mass referred to as the "crude extract." To conduct the experimental studies, the crude extract was stored in a refrigerator for further studies.

Preliminary phytochemical screening: The methanolic extract of *M. pruriens*, weighing 100 mg, was dissolved in distilled water, and the subsequent tests were conducted individually as described by Stahl [21], Harbone [22] and Wagner et al. [23].

Test for flavonoids: To the diluted extracts, a small amount of magnesium and two drops of concentrated hydrochloric acid were added, followed by gentle heating. The presence of flavonoids was indicated by the development of pink color

Test for alkaloids: Mayer's reagent was introduced to the diluted extracts. The occurrence of a cream-colored precipitate indicated the presence of alkaloids.

Test for saponins; The diluted extracts were mixed with 1 ml of water and vigorously shaken. The presence of saponins was identified by the formation of a stable froth.

Test for tannins Two milliliters of a 10% lead acetate solution were added to the diluted extracts. The presence of tannins was confirmed by the occurrence of a white color precipitate

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Test for Glycosides: The extracts underwent hydrolysis with diluted HCl for a few hours using a water bath, and the resulting hydrolysate was then subjected to Legal's and Borntrager's tests to identify the presence of glycosides. The observation of a pink color indicated the presence of glycosides.

Test for phytosterol: The extracts underwent reflux with an alcoholic potassium hydroxide solution until complete saponification occurred. The resulting mixture was then diluted and extracted with ether. The residue obtained was dissolved in a small amount of diluted acetic acid, followed by the addition of 3 ml of acetic anhydride and a few drops of concentrated sulfuric acid. The presence of phytosterol was indicated by the development of a bluish-green color.

Test for amino acids: An aliquot of the diluted extracts was combined with 2 ml of ninhydrin solution. The presence of amino acids was confirmed by the formation of a violet color.

Test for protein: For each milliliter of diluted extracts, one milliliter of 5% copper sulfate and 1% NaOH solution were introduced. The formation of a deep blue color served as an indication of the presence of protein

IN VITRO ANTIOXIDANT STUDIES

DPPH Free Radical Scavenging Activity: Scavenging of DPPH radical was assayed by the method of Ursini et al. [24]. Different concentrations of *M. pruriens* (100, 200, 300, 400, and 500 µg/ml) dissolved in methanol were prepared individually. One milliliter of the extract was mixed with an equal volume of DPPH in methanol (0.1 mM) and vigorously shaken. The mixture was then left at room temperature for 50 minutes, and the absorbance was measured at 517 nm. Ascorbic acid was used as a standard. The percentage of free radical inhibition was calculated as IC50, representing the concentration of the sample required to scavenge 50% of the DPPH free radical. The results were expressed as the percentage of inhibition per microgram of extract.

Nitric Oxide scavenging activity (NO^-): Nitric oxide is spontaneously generated by sodium nitroprusside in an aqueous solution. This nitric oxide then reacts with oxygen, resulting in the production of nitrite ions. The estimation of nitrite ions can be performed using the Griess Illosvoy reaction, as described by Garrat [25]. A reaction mixture of 3 ml was prepared, comprising sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and different concentrations of *M. pruriens* extracts (100, 200, 300, 400, and 500 µg/ml) in methanol. These mixtures were then incubated at a temperature of 25°C for a duration of two and a half hours. Following the incubation period, 0.5 ml of the reaction mixture was pipetted and combined with 1 ml of sulfanilic acid reagent. The resulting mixture was allowed to stand for 5 minutes to ensure complete diazotization. Subsequently, 1 ml of naphthyl ethylene diamine dihydrochloride was added, mixed, and left to stand at 25°C for 30 minutes. The formation of a pink-colored chromophore occurred during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine. The absorbance of the resulting solution was measured at 540 nm. The results were expressed as the percentage of inhibition per microgram of extract.

ANTIHELMINTHIC ACTIVITY:

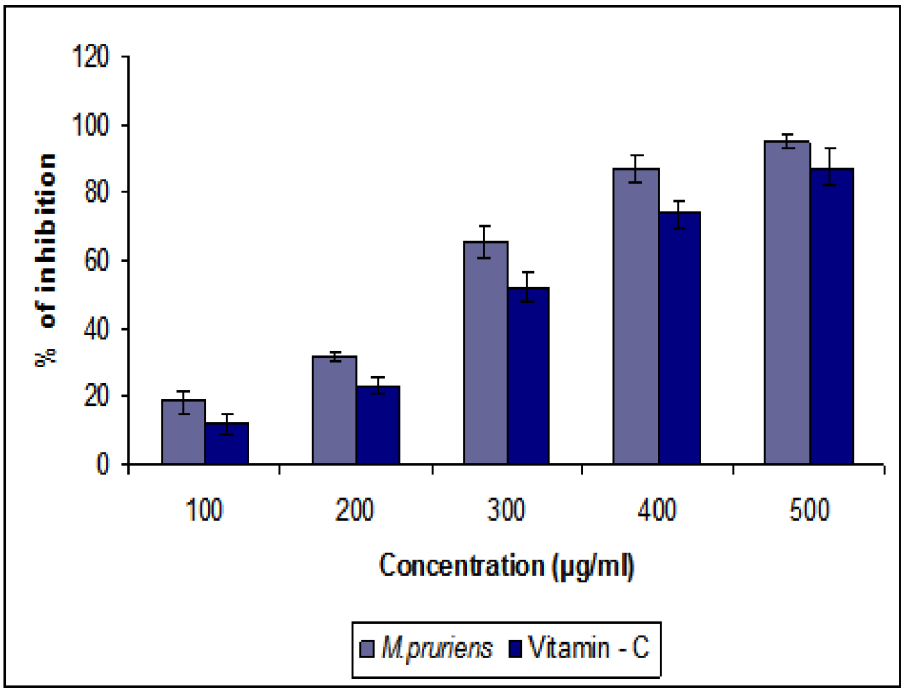

Fig. 1: Free radical scavenging activity of methanolic extract of *M. pruriens* by DPPH method. Values are mean ± SD of triplicate determination

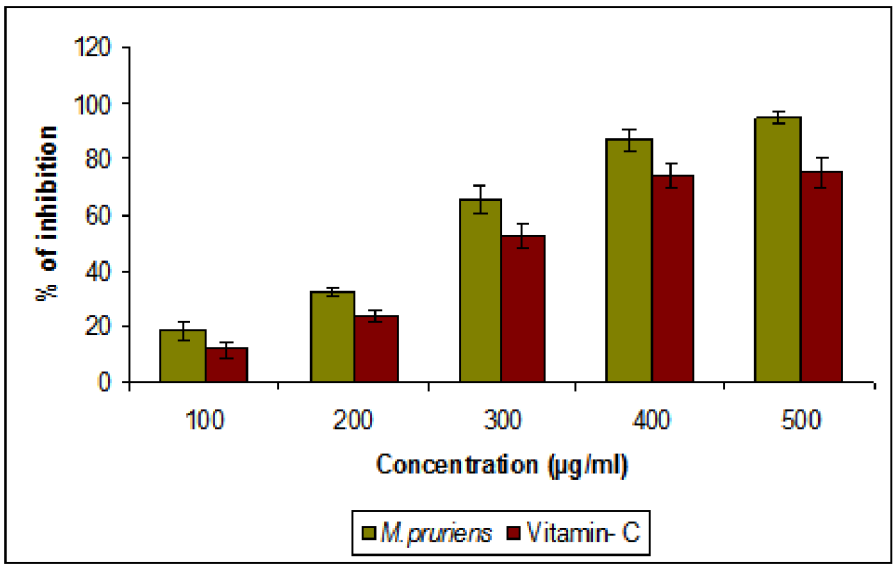

Fig. 2: Nitric oxide scavenging activity of methanolic extract of *M.pruriens*. Values are mean ± SD of triplicate determinations

Plant Constituents	Methanolic Extract
Alkaloids	Present
Saponins	Present
Tannins	Present
Phytosterol	Absent
Proteins	Present
Flavonoids	Present
Amino acids	Present
Glycosides	Present

Table 1: Phytochemical analysis of *Mucuna pruriens*

Incubation Medium	Mean mortality of *R. tetragona* at different concentrations			
	10mg/ml	20mg/ml	30mg/ml	40mg/ml
M. pruriens	3.66±0.57	2.33±0.57	2.00±1.00	2.00±1.00
Albendazole	2.12±0.15	1.40±0.17	1.00±0.32	0.62±0.5
Control	30.00±1.50			

Table 3 Antiparasitic efficacy of methanolic extract of *M. pruriens* against R.*tetragona*(invitro). Data represent mean values ± S.D of mortality (h) for five experiments.

Collection and maintenance of parasites: In the present study, we obtained living *Ascardia galli* (*A. galli*) nematode parasites and *Raillietina tetragona* cestode parasites from domestic fowl (*Gallus domesticus*). The parasites were collected using 0.9% Phosphate Buffered Saline (PBS) from a recently slaughtered host at a local market. To ensure the absence of host-related contaminants, the specimens were thoroughly rinsed in saline solution multiple times and then in distilled water. Only viable specimens were employed for the current research.

Antihelminthic activity: Nematode parasites (*A. galli*) and cestode parasites (*R. tetragona*) of consistent size were gathered and subjected to multiple washes. These parasites were then acclimatized in a phosphate buffer saline (PBS) medium with 1% glucose, pH 7.2, for a duration of 6 hours. Subsequently, the worms were transferred to a fresh PBS medium enriched with glucose (pH 7.2), containing varying concentrations of methanolic extract derived from *M. pruriens*. The survival of *A. galli* and *R. tetragona* was assessed every 2 hours, while any deceased parasites were promptly removed to prevent contamination. The concentration at which the parasite dies is referred to as the lethal concentration.

RESULTS:

The results of the initial phytochemical analysis of *M. pruriens* are summarized in Table 1. The examination of the methanolic extract of *M. pruriens* seeds revealed the presence of various compounds, including flavonoids, tannins, alkaloids, glycosides, saponins, and proteins, as determined by phytochemical analysis.

Incubation Medium	Mean mortality of *A. galli* at different concentrations			
	10mg/ml	20mg/ml	30mg/ml	40mg/ml
M. pruriens	3.00±0.00	3.00±1.00	3.00±1.73	2.00±0.00
Albendazole	2.34±0.58	1.67±0.76	1.34±0.29	0.84±0.29
Control	64.00±1.01			

Table 2: Antiparasitic efficacy of methanolic extract of *M. pruriens* against *A.galli*(invitro) Data represent mean values ± S.D of mortality (h) for five experiments

DPPH free radical scavenging activity: The DPPH method is a rapid, and convenient method that can assess the radical scavenging activity of numerous samples, regardless of their polarity [26]The DPPH method was used in the current investigation to assess the antioxidant activity of *M. pruriens*, and the results are shown in figure 1. The results reveal that the methanolic seed extract of *M. pruriens* exhibits remarkable antioxidant activity in a concentration-dependent manner at various concentrations, namely 100, 200, 300, 400, and 500 ěg/ml. Notably, at a concentration of 300 μg/ml, the *M. pruriens*seed extract demonstrates approximately 50% excellent activity, as compared to the standard vitamin C.

Nitric oxide scavenging activity: The NO scavenging activities of traditional herbal remedies were investigated in vitro using sodium nitroprusside as a NO donor. Figure 2 shows the nitric oxide scavenging activity of *M. pruriens* methanolic extract. The extract inhibits NO in a dose-dependent manner at concentrations of 100, 200, 300, 400, and 500µg/ml, based on the results of the current study. Among the levels tested, 300µg/ml of *M. pruriens* extract inhibited NO by more than 50%, which was comparable with standard vitamin C.

Antihelminthic activity: Tables 2 and 3 show the antihelminthic activity of the methanolic extract of *M. pruriens*. *A.galli* and *R. tetragona* in the control group did not show any sign of loss of physical activity and survived sturdily in the medium composed of 0.9% PBS with glucose at 37±1°C. The response in physical activity of *A. galli* and *R. tetragona* upon

treatment with Albendazole and *M. pruriens* extract found to be highly effective exhibiting profound dose-dependent activity at all concentrations. However, at lower concentration of the seed extract, the nematodes did not show significant mortality. From the present study, it is inferred that seeds of the *M. pruriens* extract exhibit remarkable Antihelminthic activity in a concentration-dependent manner.

DISCUSSION

Phytochemicals are natural compounds derived from plants that collaborate with nutrients and dietary fiber to provide protection against diseases. Numerous phytochemicals possess antioxidant properties that effectively decrease the risk of various ailments. Reactive oxygen species (ROS) have been associated with numerous diseases and the aging process. However, the presence of antioxidant systems serves to minimize or even prevent the harmful effects of ROS, safeguarding the body from their detrimental impacts [27].

Phytochemical assessment is a valuable method used to evaluate the quality of herbal drugs.In this study, the methanolic extract of *M. pruriens* seeds was first screened to identify its phytochemical composition. The analysis revealed the presence of alkaloids, tannin, protein, carbohydrates, flavonoids, saponins, and glycosides.In contrast, an earlier investigation conducted by Hadimani et al. [28] (2015) detected the lack of saponins and triterpenoids in the methanolic extract of *M. pruriens* seeds. These results correspond to the findings from a different study by Sreekala et al. [29]), where it was reported that the ethanolic extract of Mucuna seeds exhibited the presence of alkaloids, carbohydrates, flavonoids, steroids, amino acids, triterpenoids, saponin, tannin, protein, and phenol.The differences observed in the phytochemical composition can be attributed to various factors such as environmental conditions, harvesting methods, storage conditions, pesticide usage, adulteration practices, and microbial contamination. These factors contribute to significant variations in the chemical profile of medicinal plant material, as highlighted by Dhami and Mishra [30]. Verma et al. [31] further emphasized that these compounds possess antioxidant properties due to their ability to scavenge free radicals.

Natural antioxidants minimize the adverse effects of free radicals by scavenging action. It is reported that there is extensive evidence on the implication of free radicals in the development of degenerative diseases. When the generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking cellular proteins, lipids, and carbohydrates leading to the pathogenesis of many disorders such as arthritis and connective tissue, neurodegenerative, cardiovascular, reproductive disorders, diabetes, carcinogenesis and in the process of aging [32].

DPPH is used as a substrate to assess the antioxidative capability of an antioxidant compound [33]. The impact of antioxidants on DPPH radicals is believed to arise from their capacity to donate hydrogen. In this current investigation, the methanolic extract obtained from *M. pruriens* displayed an escalating scavenging effect on free radicals, which was dependent on the concentration. This outcome could be attributed to the diverse active phytochemical constituents found in the methanolic extract of *M. pruriens*. Moreover, Sanocka [34] has also proposed that a positive DPPH test serves as a reliable indicator of a plant's ability to scavenge free radicals. The therapeutic effectiveness of herbal drugs is significantly influenced by the assortment of phytochemical compounds they contain [35].

Nitric oxide holds significant importance as a bioregulatory molecule and exerts diverse physiological effects, such as regulating blood pressure, facilitating neural signal transduction, influencing platelet function, and exhibiting antimicrobial and antitumor properties [36]. It is an inorganic free radical characterized by an odd number of electrons and can form a covalent bond with other molecules by sharing a pair of electrons [37]. Nitric oxide acts as a labile intracellular messenger molecule in neurotransmission when present in low concentrations, thus executing its physiological actions. In this study, the methanolic extract derived from *M. pruriens* exhibited a suppressive effect on nitric oxide production, comparable to that of the standard vitamin C. This outcome is likely attributed to the presence of flavonoids in the extract.The present result suggest that the seed extract may be potent and novel therapeutic agents for scavenging of NO, and thereby inhibit the pathological conditions caused by excessive generation of NO and its oxidation product peroxynitrite. These findings may also help to explain the pharmacological activities like rejuvenating, anti-infection, anti-inflammatory and neuroprotective activities.

In developing countries, the cost of medicines tends to be high, leading to the utilization of medicinal plants in the development of Antihelminthic drugs [38]. In the realm of biological farming, traditional synthetic drugs are not permitted, prompting organic farmers to prefer a phytopharmaceutical approach for managing parasitic infections on their farms [39]. However, it is important to continue efforts aimed at standardizing plant extracts that exhibit potent anthelmintic activity. The goal is to develop effective alternative herbal preparations that can either replace or supplement the currently used synthetic pharmaceuticals [40].

Ascaridia galli is the predominant and significant parasite affecting poultry and wild birds, primarily infesting the intestines, and causing various health issues and pathological complications, including anemia, lethargy, weight loss, and diarrhea [41]. The primary consequence of helminthic infection is reduced absorption of nutrients, which can ultimately result in mortality [42]. In terms of effectiveness, albendazole alone exhibits efficacy against all major helminths and claims the highest cure rate [43]. Additionally, the drugs active for treating such diseases tend to be relatively costly. Given the intensifying environmental impact associated with conservative Antihelminthic treatments, it is crucial to explore alternative strategies for fighting gastrointestinal nematodes and cestodes.

To address such circumstances, native plants are utilized for the treatment of helminthic infections [44]. Numerous researchers have conducted studies to examine the Antihelmintic activity of traditional medicinal plants [45]. The effectiveness of plant materials has been assessed based on the reduction or complete elimination of spontaneous movement in *A. galli* and *R. tetragona* during in vitro experiments. In this study, an increase in the extract concentration led to the loss of movement in *A. galli* and *R. tetragona*, thereby demonstrating the antihelminthic activity of *M. pruriens*. This outcome is likely attributed to the presence of phenolic compounds in the plant extract. These findings align with the previous research by Abedulla et al. [46], who demonstrated the promising antihelminthic activity of the methanol extract of *M. pruriens* compared to the standard albendazole on *Pheretima posthuma*. Furthermore, Waller et al. [47] reported that a class of phenolic secondary metabolites has a significant impact on helminths. The results obtained from these findings are encouraging and provide a basis for further characterization and isolation of the active compound responsible for these activities.

CONCLUSION

In conclusion, *M. pruriens* has demonstrated notable in vitro antioxidant and antihelminthic properties. *M. pruriens*, an esteemed plant with historical medicinal and biochemical significance, holds considerable market value owing to its abundant array of bioactive compounds. Its antioxidant and antihelminthic properties make it highly sought after in the pharmaceutical and food industries, thereby elevating the demand for *Mucuna* in everyday life. The plant extract has exhibited strong antioxidant activity, which indicates its potential in fighting oxidative stress and associated diseases. Additionally, *M. pruriens* has displayed significant antihelminthic activity, suggesting its effectiveness in aiming and eliminating parasitic worms. These findings highlight the potential of *M. pruriens* as a valuable natural resource for the development of antioxidant and antihelminthic therapies. Further research and clinical studies are warranted to explore its full therapeutic potential and evaluate its efficacy invivo.

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