Original Article

TIPS Trends in Pharmaceutical Sciences 2024: 10(2): 121-128.

In vitro anthelmintic activity and phytochemical characterization of *Colchicum autumnale* L. corm extract

Zeenath Banu[1*];M.Pharm, Jagannadham Sharasherlin[2];B.Pharm, Aakula Nikshitha[2];B.Pharm, Jangala Akanksha[2];B.Pharm

[1]Department of Pharmacology, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkathpura, Hyderabad, India
[2]B.Pharm Students, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkathpura, Hyderabad, India

Abstract

Helminth infections are prevalent in poverty-stricken areas and developing countries with warm, humid climates and poor sanitary conditions. The limited availability of anthelmintic drugs and the emergence of drug resistance have prompted the search for new treatment options. Medicinal plants, traditionally used for treating various ailments, including parasitic infections, have been explored as sources of novel anthelmintic compounds. To evaluate the preliminary phytochemical analysis and *in vitro* anthelmintic activity of *Colchicum autumnale* L. corm extract. The anthelmintic activity of *C. autumnale* corm extract was tested against *Pheretima posthuma* (earthworms). Five different concentrations (20, 40, 60, 80, and 100 mg/ml) of the *C. autumnale* extract were used, alongside the standard drug albendazole at the same concentrations. The time taken for paralysis and death of the worms was recorded. The preliminary phytochemical qualitative analysis of the ethanolic extract showed the presence of alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, and steroids. The study highlighted the potential of *C. autumnale* extract as a natural anthelmintic agent. The dose-dependent activity suggested that higher concentrations of the extract provided more effective anthelmintic action. However, further research is necessary to establish the optimal dosing, safety, and *in vivo* efficacy of the extract.

Keywords: Helminth, *Pheretima posthuma*, *Colchicum autumnale* L., Albendazole

Please cite this article as: Banu Z*, Sharasherlin J, Nikshitha A, Akanksha J. *In vitro* anthelmintic activity and phytochemical characterization of *Colchicum autumnale* L. corm extract. Trends in Pharmaceutical Sciences. 2024;10(2):121-128. doi: 10.30476/tips.2024.103051.1246

1. Introduction

Parasitic worms, also known as helminths, are multicellular organisms that can infect humans and animals, causing a range of diseases known as helminthiasis. These worms belong to different classes, including nematodes (roundworms), cestodes (tapeworms), and trematodes (flukes). They typically infect the gastrointestinal tract, but some species can also affect other organs such as the liver, lungs, and blood vessels (1). In humans, parasitic worm infections contribute to a global disease burden of approximately 14 million cases, categorized under neglected tropical diseases (NTDs). These diseases disproportionately affect populations in low-resource regions, where access to healthcare and sanitation is limited. In agricultural animals, parasitic worm infections result in substantial economic losses, amounting to billions of dollars annually worldwide. These losses arise from decreased productivity, including reduced growth rates, milk and meat production, and reproductive efficiency, as well as expenditures on treatment and prevention measures (2, 3). Helminths reside within the gastrointestinal (GI) tract of their

Corresponding Author: Zeenath Banu, Department of Pharmacology, RBVRR Women's College of Pharmacy, Affiliated to Osmania University, Barkathpura, Hyderabad, India
Email: zeenathcology@gmail.com

Received: 13/04/2024; Accepted: 01/06/2024

hosts, deriving sustenance and causing infection or disease by feeding off living host tissues and nutrients. This parasitic relationship predominantly affects children, with soil-transmitted schistosomiasis and helminthiasis being the most prevalent among neglected tropical diseases (1, 4). Furthermore, helminth infections contribute to an indirect disease burden by impairing the immune system, thus exacerbating the susceptibility to other infectious diseases such as malaria, tuberculosis, and human immunodeficiency virus/acquired immunodeficiency syndrome (5). Adult parasites have a prolonged lifespan within their human hosts, directly extracting nutrients from the host's blood, consequently leading to conditions such as iron deficiency anaemia. Anthelmintic drugs are the primary treatment option for helminth infections, but their efficacy is threatened by emerging drug resistance and limited availability in resource-constrained settings. Natural products, including plant extracts, have shown promise as potential sources of novel anthelmintic agents. Therefore, exploring plant-based compounds can be crucial in identifying targets for anthelmintic drug development. Similarly, *C. autumnale* has been selected for studying its anthelmintic activity.

Colchicum autumnale L., commonly known as autumn crocus or meadow saffron, is a perennial herbaceous plant native to Europe and Western Asia. It belongs to the family Colchicaceae. The plant typically grows from a corm and produces pink to purple flowers in the autumn, hence its common name. The plant contains colchicine, an alkaloid known for its potent anti-inflammatory and analgesic effects. Additionally, colchicine's antimitotic properties, attributed to its interference with microtubule formation, have been exploited in cancer therapy (6). To date, there are no reports demonstrating the anthelmintic activity of the corm of *C. autumnale*. Hence, the current study aims to evaluate the anthelmintic activity of *C. autumnale* corm using experimental models.

2. Material and methods

2.1 Plant material

C. autumnale corms were collected from Yucca Enterprises, Mumbai, India, in April 2024. The dried bulbs were ground to a particle size of 80-100 mesh before extraction. The plant material was authenticated by Dr. Vijaya Bhasker Reddy, Assistant Professor in the Department of Botany at Osmania University, with the voucher number OUAS-206.

2.2 Extraction protocol

The powdered plant material (100g) of *C. autumnale* was extracted with 80% ethanol at a temperature of 55 to 60 °C for 48 h using a Soxhlet apparatus. The resulting extract was concentrated using a rotary evaporator and then stored in a refrigerator at 4 °C until further use.

2.3 Drugs and chemicals

All chemicals and reagents were obtained from HI Media (Mumbai, India) and were of analytical grade. The drug Albendazole was collected from GlaxoSmithKline Pharmaceuticals Limited.

2.4 Phytochemical screening

Qualitative phytochemical screening of the ethanolic extract of *C. autumnale* was conducted to detect the presence of bioactive compounds such as alkaloids, flavonoids, tannins, carbohydrates, and saponins, following standard protocols (7). The results, depicted in Table 1, indicated the presence of various phytochemicals. Alkaloids were detected using Dragendorff's reagent, and Mayer's reagent which forms coloured precipitates. Flavonoids were identified using the Shinoda test, where the addition of magnesium and hydrochloric acid produced a pink or red colouration. Tannins were detected with the Ferric Chloride test, which produced a blue-black or green-black colouration. Saponins were identified using the froth test, where vigorous shaking with water produced stable froth. Steroids and terpenoids were detected using the Salkowski test, where chloroform and concentrated sulfuric acid yielded a reddish-brown colour. Phenolic compounds were detected with the ferric chloride test, producing a blue or green colour (7).

2.5 Experimental model

Pheretima posthuma is a species of earthworm commonly found in moist soil environments. These earthworms were carefully selected and collected for the purpose of conducting an anthelmintic drug study. Before the experiment, the earthworms underwent a cleaning process using a normal saline solution to remove any dirt or de-

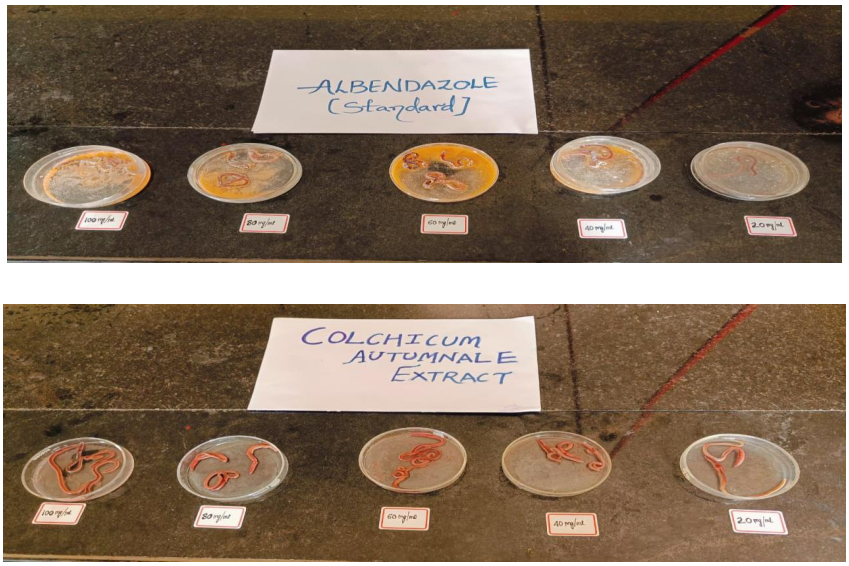

Figure 1. Anthelmintic activity of Albendazole and *C. autumnale* extract.

bris from their surface. The choice of *P. posthuma* as a model organism for this study was based on several factors. Firstly, these earthworms share significant anatomical and physiological similarities with human intestinal roundworm parasites, such as their digestive system and neuromuscular structure. This makes them an appropriate model for evaluating the efficacy of anthelmintic drugs. Secondly, *P. posthuma* is readily accessible and can be easily collected from their natural habitat, making them a convenient and cost-effective option for laboratory studies (8,9).To ensure the reliability and reproducibility of the experiment, the earthworms were divided into two groups, each consisting of three individuals. The earthworms within each group were selected to be of approximately equal size to minimize any potential variations in drug response due to size differences.

2.5.1 Anthelmintic evaluation

Albendazole was used as the standard drug, and its concentrations were prepared by dissolving it in DMSO to achieve concentrations of 20 mg/ml, 40 mg/ml, 60 mg/ml, 80 mg/ml and 100 mg/ml.

2.5.2 Anthelminthic assay

In the present study, the experimental design involved two groups, each consisting of approximately three earthworms of similar size, measuring 10 cm in length. These groups were treated with varying concentrations of Albendazole and ethanolic extract solutions, ranging from 20 mg/ml to 100 mg/ml, in increments of 20 mg/ml. The test solution (ethanolic extract) and the standard drug solution (Albendazole) were prepared fresh to ensure their potency and effectiveness.

Throughout the experiment, close observations were made to record the time taken for paralysis and death of individual worms in each group. Paralysis was defined as the state in which the worms failed to revive or show any movement, even when placed in a normal saline solution. This criterion was used to determine the onset of paralysis in the earthworms. The time of death was established when the worms exhibited no movement upon vigorous shaking or immersion in slightly warm water. This step was crucial in assessing the lethal effect of the tested compounds on the earthworms. End Point: The time of paralysis and death of the worms were determined (10, 11).

2.5.3 Statistical analysis

To analyse the data, the mean and standard error of the mean (SEM) were calculated for each group. These values were then subjected to statistical analysis using the student t-test, with the level of significance set at $p < 0.05$.

3. Results

3.1. Percentage yield of ethanolic extract of C. autumnale corm

Table 1. Phytochemical analysis of *C. autumnale* corm extract.

Phytochemical	*C. autumnale* extract
Alkaloids	+
Tannins	+
Steroid	+
Phenols	+
Carbohydrates	+
Glycosides	+
Saponins	+
Amino acid	-
Fats and oil	-

(+) =Presence; (-) =Absence.

The percentage yield was calculated using the formula below and was determined to be 2%.

3.2. Preliminary phytochemical screening

The phytochemical analysis of the ethanolic extract, as presented in Table 1, revealed the presence of various bioactive compounds, including alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, and steroids. These findings provide valuable insights into the potential mechanisms behind the observed anthelmintic activity of the extracts (Equation 1).

$$\%Dry\ weight = \frac{weight\ of\ dry\ sample}{weight\ of\ the\ sample} \times 100$$
$$= \frac{2}{100} \times 100 = 2\% \qquad \text{(Eq. 1)}$$

3.3. Anthelmintic activity of C. autumnale extract and standard drug

The study evaluated the anthelmintic activity of the ethanolic extract of *C. autumnale* using *Pheretima posthuma* worms as the test organism. The results demonstrated a dose-dependent relationship between the concentration of the extract and its anthelmintic efficacy. As the concentration of the extract increased from 20 mg/mL to 100 mg/mL, the time required for paralysis and death of the worms decreased significantly. At the lowest concentration of 20 mg/mL, the extract induced paralysis in the worms at 33.33 min and death at 59.08 min. As the concentration increased to 40, 60, 80, and 100 mg/mL, the paralysis time reduced to 27.54, 22.31, 14.94 and 13.51 min, respectively. Similarly, the death time decreased to 50.76, 38.3, 29.85, and 23.68 min at these higher concentrations (Table 2). In comparison, the standard drug albendazole, used as a positive control, exhibited faster paralysis and death times at the same concentrations. At 20, 40, 60, 80, and 100 mg/mL, albendazole induced paralysis in 15.36, 12.21, 11.97, 10.81, and 10.63min, and death in 16.11, 16.86, 14.67, 12.36, and 11.67 min, respectively.

4. Discussion

Plant-derived natural products have emerged as promising reservoirs of novel therapeutic agents. Their medicinal properties have garnered attention due to their potent pharmaco-

Table 2. Anthelminthic assay of *C. autumnale* against *P. posthuma*.

Drug	Concentration (mg/ml)	Paralysis time (min) (Mean ± SEM)	Death time (min) (Mean ± SEM)
Albendazole	20	15.36 ± 0.12	16.11 ± 0.28
	40	12.21 ± 0.02	16.86 ± 0.90
	60	11.97 ± 0.35	14.67 ± 0.32
	80	10.81 ± 0.40	12.36 ± 0.06
	100	10.63 ± 0.24	11.67± 0.16
Ethanolic extract of *C. autumnale*	20	33.33 ± 0.57	59.08 ± 0.78
	40	27.54 ± 0.32	50.76 ± 0.95
	60	22.31 ± 0.52	38.31 ± 1.11
	80	14.94 ± 0.37	29.85 ± 2.44
	100	13.51 ± 1.33	23.68 ± 0.33

Each value in the table is represented as mean ± SEM (n = 3). Comparisons made between standard versus treated group, $p < 0.05$ was considered significant.

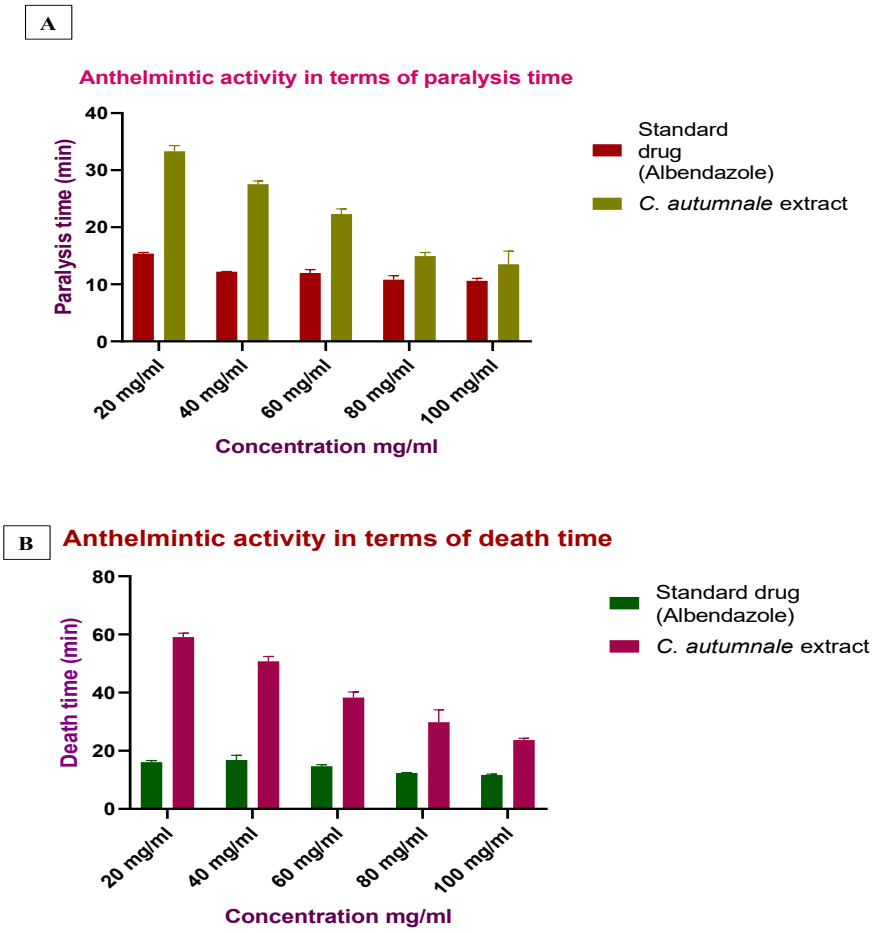

Figure 2. Anthelmintic activity (A) Paralysis time (B) Death Time.

logical activities, relatively low toxicity profiles, and economic viability. Notably, a significant proportion of clinically active drugs originate from natural sources, underscoring the importance of natural products in the drug discovery process. Therefore, investigating medicinal plants is essential for identifying active natural product ingredients that could potentially be utilized for disease management. Subsequently, these identified active ingredients may undergo laboratory synthesis for further exploration and development. Helminth infections pose a significant challenge for both humans and animals, often resulting in chronic and severe diseases that can lead to fatalities. Additionally, these infections can contribute to the development of drug resistance in other diseases. Addressing this issue requires research efforts centred on exploring natural products as potential preventatives against helminth infections (12).

In the present study, the anthelmintic activity of the ethanolic extract of *C. autumnale* was evaluated by measuring the time taken for paralysis and death of individual worms. The results were compared to the standard drug albendazole, which served as a positive control. Albendazole is a widely used broad-spectrum anthelmintic drug that acts by interfering with the microtubule structure of the parasite. It disrupts the cytoplasmic microtubules, which are essential for various cellular processes, including glucose uptake and ATP generation. By impairing the parasite's energy metabolism, albendazole leads to the immobilization and eventual death of the worms. Interestingly, if the worms are removed from the albendazole-containing medium, they may recover, indicating the reversibility of the drug's effects (13).

The results of the current study suggest that the ethanolic extract of *C. autumnale* exhibits

significant anthelmintic activity in a dose-dependent manner. As the concentration of the extract increased, the time taken for paralysis and death of the worms decreased, indicating a stronger anthelmintic effect at higher doses. The observed anthelmintic activity of the *C. autumnale* extract can be attributed to the presence of bioactive phytoconstituents, such as alkaloids, carbohydrates, glycosides, saponins, phenols, tannins, and steroids. These compounds have been reported to possess various pharmacological properties, including anthelmintic activity. Alkaloids may impact the central nervous system, potentially causing paralysis. They include steroidal alkaloids and oligoglycosides, which can inhibit the transfer of sucrose from the stomach to the small intestine. Additionally, alkaloids exhibit antioxidant properties, reducing nitrate generation and thereby affecting local homeostasis essential for helminth development (14). Although carbohydrates are not typically recognized for their direct anthelmintic activity, certain complex carbohydrates and polysaccharides can influence parasitic infections through indirect mechanisms. For example, polysaccharides from plant extracts, such as those from *Glycyrrhiza glabra* (licorice), can modulate the host's immune response, enhancing its ability to combat helminth infections. Additionally, some carbohydrates may interfere with the adhesion of parasites to host tissues, disrupting the attachment of worms to the intestinal wall (15). Glycosides, which consist of a sugar moiety attached to a non-sugar component (aglycone), have demonstrated anthelmintic properties through various mechanisms. Saponins, a type of glycoside found in plants like *Quillaja saponaria* (soap bark), can damage the cell membranes of parasites, leading to their death. Glycosides may also interfere with the metabolic processes of helminths; for instance, cardiac glycosides can disrupt the ion balance of parasitic worms, affecting their viability. Saponins affect the permeability of parasite cell membranes, causing vacuolization and disintegration of their teguments (16). Phenolic compounds disrupt the energy generation mechanisms in parasites by uncoupling oxidative phosphorylation. They also interfere with the glycoproteins on the surface of parasite cells, leading to their death (17). Tannins affect the energy generation of worms by uncoupling oxidative phosphorylation. They may also bind to free proteins in the gastrointestinal tract of the host or to glycoproteins on the worms' cuticles, leading to the parasites' death (18). Steroids exert anthelmintic effects by impacting cellular processes in parasites. Steroidal compounds, such as those in *Datura stramonium* (jimson weed), can integrate into parasite cell membranes, causing structural damage and functional disruption. Steroids can also influence the hormonal regulation within helminths, disrupting their growth and reproduction by affecting their endocrine system.

C. autumnale is utilized in the Unani system of medicine for treating worm infestations through specific formulations. Notably, it is used in the preparation of Kirm-e-Shikam, which targets intestinal worms, and Habb-al-Qara, which is formulated to address tapeworm infections (19). These traditional remedies leverage the therapeutic properties of *C. autumnale* to effectively manage and combat parasitic worm infestations. The primary active ingredient in *C. autumnale* that accounts for its pharmacological effects is colchicine. Colchicine is well-known for its various biological activities, including its anti-inflammatory and anthelmintic effects. It works by disrupting the function of microtubules within cells. Microtubules are critical for several cellular processes, such as cell division and intracellular transport, which are vital for the survival and reproduction of helminths (20). The findings of this study highlight the potential of *C. autumnale* extract as a natural anthelmintic agent. The dose-dependent activity suggests that higher concentrations of the extract may provide more effective anthelmintic action. However, further studies are necessary to determine the optimal dose range and to evaluate the safety and efficacy of the extract *in vivo*.

5. Conclusion

This study highlights the potential of *C. autumnale* extract as a natural anthelmintic agent, demonstrating significant activity in a dose-dependent manner. The presence of various bioactive compounds likely contributes to its efficacy. These findings suggest that *C. autumnale* extract could be a viable alternative to conventional drugs like

albendazole for managing helminth infections. Further research is needed to optimize dosing and assess safety and effectiveness *in vivo*.

Acknowledgements

The authors are thankful to Prof M. Sumakanth, Principal and Prof. J. Archana, HOD, Department of Pharmacology, RBVRR Women's College of Pharmacy for providing the necessary facilities to carry out this research work.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. *J Clin Invest.* 2008 Apr;118(4):1311-21. doi: 10.1172/JCI34261. PMID: 18382743; PMCID: PMC2276811.
2. Fenwick A. The global burden of neglected tropical diseases. *Public Health.* 2012 Mar;126(3):233-236. doi: 10.1016/j.puhe.2011.11.015. Epub 2012 Feb 9. PMID: 22325616.
3. Knox MR, Besier RB, Le Jambre LF, Kaplan RM, Torres-Acosta JF, Miller J, Sutherland I. Novel approaches for the control of helminth parasites of livestock VI: summary of discussions and conclusions. *Vet Parasitol.* 2012 May 4;186(1-2):143-9. doi: 10.1016/j.vetpar.2011.11.054. Epub 2011 Nov 20. PMID: 22154257.
4. World Health Organization. Soil-transmitted helminth infections [Internet]. Geneva: World Health Organization; 2022 [cited 2024 Aug 13]. Available from: https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections
5. Hotez P, Whitham M. Helminth infections: A new global women's health agenda. Obstetrics & Gynecology. 2014 Jan 1;123(1):155-60.
6. Dalbeth N, Lauterio TJ, Wolfe HR. Mechanism of action of colchicine in the treatment of gout. *Clin Ther.* 2014 Oct 1;36(10):1465-79. doi: 10.1016/j.clinthera.2014.07.017. Epub 2014 Aug 21. PMID: 25151572.
7. Khandelwal KR. Practical pharmacognosy. 1st ed. Pune: Nirali Prakashan; 1995;149-155.
8. Das SS, Dey M, Ghosh AK. Determination of anthelmintic activity of the leaf and bark extract of tamarindus indica linn. *Indian J Pharm Sci*. 2011 Jan;73(1):104-7. doi: 10.4103/0250-474X.89768. PMID: 22131633; PMCID: PMC3224400.
9. Paul, A., Adnan, M., Majumder, M. et al. Anthelmintic activity of Piper sylvaticum Roxb. (family: Piperaceae): In vitro and in silico studies. *Clin Phytosci.* 2018;4:17.
10. Hasan K, Lakshmi T, Rathinam TK. Preliminary Phytochemical Analysis and In vitro Anti-helmenthic activity of Achyranthes aspera Leaf extract. *Phcog J.* 2015;7(6):397-399.
11. Ishnava KB, Konar PS. In vitro anthelmintic activity and phytochemical characterization of Corallocarpus epigaeus (Rottler) Hook. f. tuber from ethyl acetate extracts. *Bull Natl Res Cent.* 2020 Dec;44:1-10.
12. De Rycker M, Baragaña B, Duce SL, Gilbert IH. Challenges and recent progress in drug discovery for tropical diseases. *Nature*. 2018 Jul;559(7715):498-506. doi: 10.1038/s41586-018-0327-4. Epub 2018 Jul 25. PMID: 30046073; PMCID: PMC6129172.
13. Malik K, Dua A. Albendazole. 2023 Apr 10. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan–. PMID: 31971723.
14. Roy H, Chakraborty A, Bhanja S, Nayak BS, Mishra SR, Ellaiah P. Preliminary phytochemical investigation and anthelmintic activity of Acanthospermum hispidum DC. *J Pharm Sci Technol.* 2010;2(5):217-21.
15. Wu Y, Wu C, Che Y, Zhang T, Dai C, Nguyễn AD, Duan K, Huang Y, Li N, Zhou H, Wan X, Wang Y, Lei H, Hao P, Li C, Wu Y. Effects of Glycyrrhiza Polysaccharides on Chickens' Intestinal Health and Homeostasis. *Front Vet Sci.* 2022 May 12;9:891429. doi: 10.3389/fvets.2022.891429. PMID: 35647094; PMCID: PMC9134109.
16. Melzig MF, Bader G, Loose R. Investigations of the mechanism of membrane activity of selected triterpenoid saponins. *Planta Med.* 2001 Feb;67(1):43-8. doi: 10.1055/s-2001-10632. PMID: 11270721.
17. John J, Mehta A, Shukla S, Mehta P. A report on anthelmintic activity of Cassia tora leaves. *Songklanakarin J Sci Technol.* 2009 May 1;31(3):269-271.

18. Patel J, Kumar GS, Qureshi MS, Jena PK. Anthelmintic activity of Ethanolic extract of whole plant of Eupatorium Odoratum. L. *Int J Phytomed*. 2010;2(2):127-132.
19. Khan MA. Muheet-i Azam. Vol. 1. New Delhi: Central Conservative Research and Training Institute; 2014:194-197.
20. Leung YY, Yao Hui LL, Kraus VB. Colchicine--Update on mechanisms of action and therapeutic uses. *Semin Arthritis Rheum.* 2015 Dec;45(3):341-50. doi: 10.1016/j.semarthrit.2015.06.013. Epub 2015 Jun 26. PMID: 26228647; PMCID: PMC4656054.