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Evaluation of the anthelmintic activity of ethanol leaf extract of *Dimocarpus longan* (Lour.) against *Taenia solium*

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Abstract
Helminth infections affects nearly 1.5 billion people worldwide, particularly in tropical and subtropical regions, where poverty, inadequate housing, and poor sanitation, exacerbate their impact. These infections pose significant health and economic burdens, and the limited effectiveness of current anthelmintic drugs underscores the need for alternative treatments. *Dimocarpus longan*, traditionally used in various forms of medicine, is rich in bioactive compounds such as flavonoids, tannins, and saponins, which are known to exhibit antiparasitic properties. This study evaluated the anthelmintic potential of ethanol leaf extract of *D. longan* against *Taenia solium*, aiming to explore its use as a natural remedy for managing helminth infections. The extract, tested at concentrations of 25, 50, 100, and 200 mg/mL. alongside piperazine citrate (10 mg/mL) on adult *T. solium* worms obtained from freshly slaughtered pigs. The anthelmintic activity was measured by recording the time to paralysis and death of the worms. The extract showed significant concentration-dependent effects, with higher concentrations (100 & 200 mg/mL) demonstrating efficacy comparable to piperazine citrate, exhibiting strong paralytic but weaker lethal effects on *T. solium*. The findings suggest that *D. longan* ethanol leaf extract has potent anthelmintic properties, making it a promising natural alternative for managing helminth infection.

Keywords: Anthelmintic activity; *Dimocarpus longan*; *Taenia solium*; Cestodes; Piperazine citrate

INTRODUCTION

Helminths are large macro-parasites characterized by elongated, flat, or round bodies that are visible to the naked eye in their adult forms. Most helminths are intestinal worms that primarily infect the gastrointestinal tract, often through soil-transmitted or ingested forms, although some species also affect the musculoskeletal system [1]. Helminth infections pose a significant public health concern, particularly in tropical and subtropical regions, where they challenge global health and economic stability [2,3]. The World Health Organization (WHO) estimates that nearly 1.5 billion people globally are infected with helminths, representing almost a quarter of the world's population, leading to an estimated 5.19 million disability-adjusted life years (DALYs) [4]. The prevalence of these

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infections is closely linked to severe poverty, substandard housing, and poor sanitation [5].

Infections are especially common in Africa, Latin America, and Asia, where helminths such as *Taenia solium* affect both human and animal hosts, contributing to financial losses and disease burden. For instance, the prevalence of taeniasis and human cysticercosis ranges from 2.3% to 5.2%, with human cysticercosis exceeding 16% in some areas [6]. These parasites exert a dual impact by infecting both intermediate and definitive hosts, resulting in financial losses, mortalities, morbidities, and social stigma [6]. Neurocysticercosis, a severe form of the disease, is a leading cause of epilepsy in adults [7]. In sub-Saharan Africa, tapeworms, including *T. solium*, are among the most common helminth infections, affecting both human and animal populations. The four main human tapeworms include *Taenia solium* (pork tapeworm), *Taenia saginata* (beef tapeworm), *Diphyllobothrium latum* (fish tapeworm), and *Hymenolepis nana* (dwarf tapeworm) [8].

In Uganda, the prevalence of porcine cysticercosis has increased significantly, rising from 9% in 2005 to 26% in 2021. This surge is partly attributable to Uganda's status as one of Africa's largest pig producers, with the pig population growing from 3.18 million in 2008 to 4.5 million in 2018. Additionally, Uganda is one of the largest consumers of pork in sub-Saharan Africa [9,10]. Globalization has facilitated the spread of *T. solium* taeniasis/cysticercosis (TSTC) across borders, underscoring the importance of coordinated control efforts. Despite ongoing interventions such as deworming campaigns, health education, and sanitation initiatives, sub-Saharan Africa still lacks comprehensive control programs, making the need for alternative treatments critical [11]. Helminth infections can cause a range of health issues, including gastroenteritis, anemia, stunted growth, poor nutritional status, and reduced physical and intellectual abilities [12].

Anthelmintics are agents used to expel parasitic worms (helminths) from the body by either stunning or killing them. While current anthelmintic drugs such as benzimidazole and albendazole are effective, they have limitations, including the development of resistance and potential side effects [13,14]. Resistance to these drugs occurs due to prolonged use, leading to reduced efficacy and posing a major challenge in helminth control efforts. Consequently, there is a growing need to explore alternative therapies, including plant-based anthelmintics. Medicinal plants offer a rich source of botanical anthelmintic remedies; thorough evaluation and screening of these plants could provide sustainable and environmentally friendly alternatives to synthetic drugs [1].

Dimocarpus longan, commonly known as longan or *omuhingura* by the Runyankole-speaking tribe in Western Uganda, is a tropical and subtropical evergreen tree traditionally used for various therapeutic purposes, including anthelmintic properties. The longan tree can reach up to 20 meters in height and features mild green leaves, unisexual/bisexual flowers, and heart-shaped fruits [15].

Due to its rich chemical composition, *D. longan* is considered a potential alternative source of medication for a wide range of diseases. Various parts of the plant have effectively been used to treat conditions such as amnesia, bone fractures, chickenpox, cuts and wounds, fever, gastrointestinal disorders, insomnia, menstrual problems, nerve pain, neurological disorders, snake bites, and reproductive health issues [15]. Bioactive compounds, such as tannins, alkaloids, flavonoids, and phenolics, are known for their diverse biological activities, which include anti-parasitic effects, making *D. longan* an attractive candidate for further research in helminth control. As a crude drug, *D. longan* exhibits numerous biological activities, including anti-proliferative, antioxidant, anti-cancer, anti-tyrosinase, radical scavenging, anti-inflammatory, anti-microbial, osteoblast differentiation activation, anti-fungal, immunomodulatory, probiotic, anti-aging, anti-diabetic, obesity management, and suppressive effects on macrophage cells [16].

The acute toxicity profile (20 g/kg) and chronic toxicity studies (5 g/kg for 180 days) in rats showed that the plant is safe for oral consumption, as no physical, hematological, serum biochemical, or histopathological changes of clinical concern were observed [17]. Given the limitations of existing anthelmintic treatments and the rich medicinal properties of *D. longan*, this study aims to explore its ethanol leaf extract as a potential natural alternative for managing helminth infections.

EXPERIMENTAL METHODS

Collection of leaves of *D. longan*. The leaves of *D. longan* were collected from Bwegiragye village in Ishaka-Bushenyi, Uganda, in September 2023. Dr. Olet Eunice from the Department of Botany, Mbarara University of Science and Technology, Uganda, identified and authenticated the leaf samples, and a voucher specimen (TF00912) was deposited. The leaves were shade-dried under controlled conditions, maintaining a temperature of approximately 25°C and relative humidity below 60% to prevent fungal growth until fully dried. Regular turning of the leaves was performed to ensure even drying. Once dried, the leaves were milled into a coarse powder using a blender and stored in an airtight container for future use. This powdering process was crucial to enhance the dissolution of phytochemicals during the maceration process.

Extraction. The extraction process was carried out using the maceration method with 70% ethanol as the solvent. One hundred grams of air-dried leaf powder was immersed in 500 mL of 70% ethanol in a conical flask and kept at room temperature for three days, with occasional shaking. On the fourth day, the mixture was filtered through a muslin cloth and filter paper [18]. The filtrate was then evaporated using a water bath maintained at 60°C to obtain a concentrated extract. This extract was used to prepare suspensions of 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL of crude ethanolic extracts of *D. longan* leaves in normal saline [19].

Extract yield. The percentage yield of the extract was obtained after extraction using the formula;

$$\text{Percentage yield (\%)} = \frac{weight\ of\ extract}{Weight\ of\ powder\ leaf} X\ 100$$

Collection of worms. Adult tapeworms were harvested from the intestines of freshly slaughtered pigs at the Ishaka-Kabirizi pork joint. To ensure standardization, only healthy adult tapeworms of similar size (approximately 10-15 cm in length) were selected for the study. The worms were thoroughly washed in Petri dishes containing 0.9% neutral phosphate-buffered saline (PBS), which was maintained at 37°C in a microbiological incubator.

Standard drug used. Piperazine citrate, at a concentration of 10 mg/mL, was used as the standard drug and prepared freshly for each experiment. Normal saline plus phosphate-buffered saline served as the normal control [20, 21].

Anthelmintic activity. The anthelmintic activity was assessed according to the method described by Das [21] on *Taenia solium* of approximately equal size, which were randomly selected for the study [18]. The tapeworms were divided into six groups, each containing six worms. Piperazine citrate at a concentration of 10 mg/mL served as the standard, while the test extract was prepared at concentrations of 25 mg/mL, 50 mg/mL, 100 mg/mL, and 200 mg/mL. Phosphate-buffered saline was used as the control. Six tapeworms were exposed to each concentration and placed in Petri dishes at room temperature. The time taken for complete paralysis and death was recorded, and the mean paralysis time and mean lethal time for each sample were calculated.

Determination of time of paralysis and time of death. Paralysis time was recorded when the worms showed no movement, except when vigorously shaken, with a predefined threshold of no observable movement for at least 30 seconds. The time of death, in minutes, was noted after confirming that the worms remained immobile even after vigorous shaking for the same duration. Worms with no observable motility were removed; if they revived upon shaking, they were counted as alive; otherwise, they were considered dead [3]. The worms were observed and recorded at 10-minute intervals throughout the experiment to ensure that no events were missed.

Statistical analysis. Data from the anthelmintic activity tests were analyzed statistically using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test to identify significant differences between the groups. Results were expressed as mean ± Standard Error of the Mean (SEM) for each group. All statistical analyses were conducted using GraphPad Prism software version 8, with a significance level set at $p \leq 0.05$.

Ethical consideration. Ethical approval was obtained from the Institutional Research and Ethics Committee for Biosafety (IREC) of Kampala International University-Western Campus (Number: KIU-2023-09). Chemical wastes were disposed of as protocol by the Kampala International University Pharmacy Laboratory guideline.

RESULTS

Extract yield. The percentage yield of the ethanol leaf extract of *D. longan* was 19%.

Paralysis. The ethanol leaf extract of *D. longan* induced paralysis in the worms at higher concentrations of 100 mg/mL and 200 mg/mL at 32 and 22 minutes, respectively. These effects were comparable to the standard drug, piperazine (p-value = 0.083 and 0.125, respectively) which caused paralysis at 23 minutes. In contrast, the lower concentrations, 25 mg/mL and 50 mg/mL, resulted in paralysis at 52 and 40 minutes, respectively, which were significantly different from the standard drug, with p-values of ≤ 0.01 and ≤ 0.001, respectively (Figure 1).

Vermicidal. The ethanol leaf extract of *D. longan* resulted in worm deaths occurring between 100 to 150 minutes across all concentrations. These times were significantly different from those observed with the standard drug, with p-values of ≤ 0.01, ≤ 0.001, and ≤ 0.0001 (Figure 2).

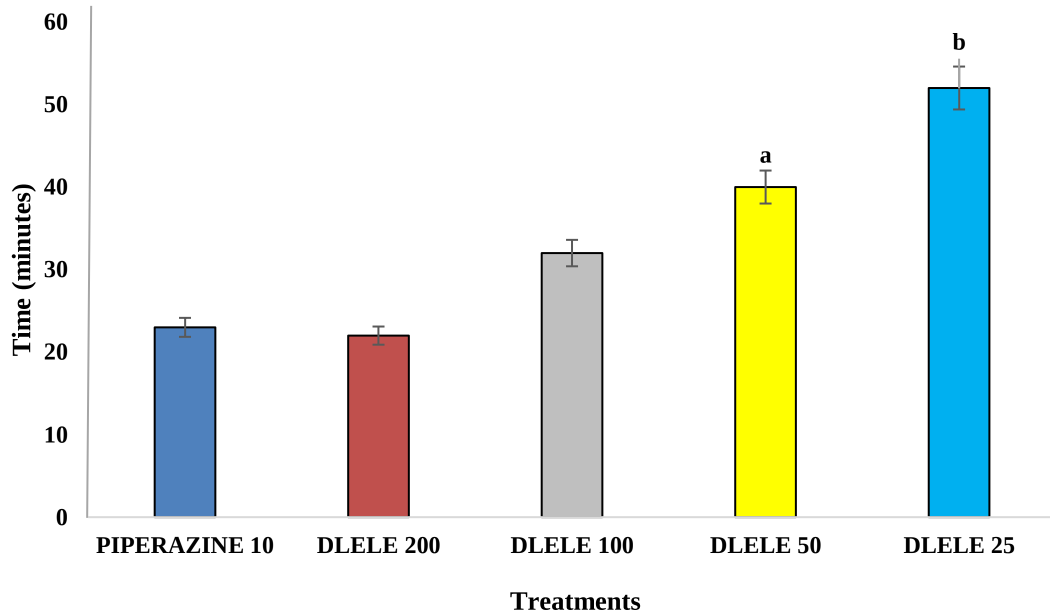

Figure 1: Anthelmintic activity (paralysis) of ethanol leaf extract of *Dimocarpus longan* against *Taenia solium.* a= $p \leq 0.01$, b= $p \leq 0.001$ significantly different from Piperazine; DLELE = *Dimocarpus longan* ethanol leaf extract.

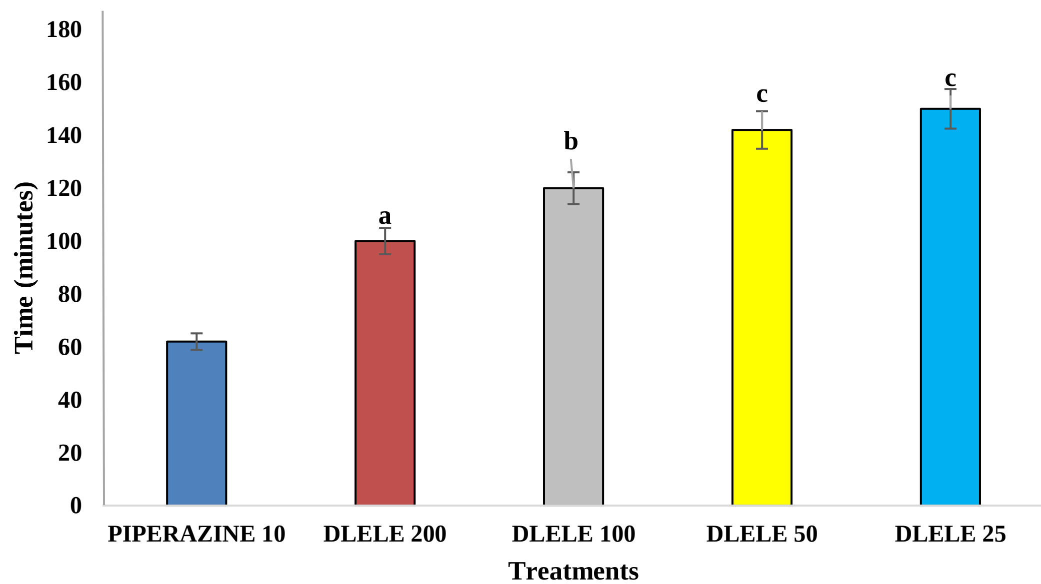

Figure 2: Anthelmintic activity (death) of ethanol leaf extract of *Dimocarpus longan* against *Taenia solium.* a= $p \leq 0.01$, b= $p \leq 0.001$, c= $p \leq 0.0001$significantly different from Piperazine; DLELE = *Dimocarpus longan* ethanol leaf extract.

DISCUSSION

Helminth infections represent a major and widespread global health burden, particularly in developing countries, where they frequently cause acute and chronic illnesses, leading to substantial morbidity and mortality [22]. Addressing this public health challenge necessitates the discovery of new and effective anthelmintics. *D. longan*, a plant with a long history of traditional medicinal use, has been explored for its potential anthelmintic properties against tapeworms, a type of helminth parasite.

Ethanol has been identified as the most effective solvent for extracting *D. longan* [23]. The percentage yield of the ethanol leaf extract was 19%. The yield of an extract provides insights into the efficiency of the extraction procedure. Higher yields typically indicate a more effective extraction method, as more of the desired compounds are extracted from the plant material [24].

The ethanol extract of *D. longan* has demonstrated a dose-dependent paralytic and lethal effect on tapeworms, with higher concentrations demonstrating enhanced activity, suggesting its significant anthelmintic potential [25]. At higher concentrations, the extract's paralytic efficacy was comparable to that of piperazine citrate, a standard anthelmintic drug, highlighting its potential as a therapeutic agent. Piperazine works by acting as a GABA (gamma-aminobutyric acid) receptor agonist in worms. It binds to GABA receptors in the muscle cells of the worms, increasing chloride ion conductance. This process leads to hyperpolarization of the muscle cell membranes, resulting in muscle relaxation and flaccid paralysis. Consequently, the paralyzed worms lose their grip on the intestinal wall and are expelled from the host's body through the natural movement of the intestines known as peristalsis [26].

The leaf extract of *D. longan* contains diverse bioactive compounds, such as tannins, phenolic compounds, alkaloids, saponins, and flavonoids, which contribute to its anthelmintic activity [27-30]. Tannins, for instance, are known to precipitate proteins and disrupt the physiological processes of helminths, leading to their immobilization and eventual death. Phenolic compounds, on the other hand, possess antioxidant properties that may enhance the plant's overall therapeutic effects and could also interfere with the metabolic activities of the worms [31, 32]. This finding aligns with previous research on medicinal plants like Artemisia, Iris, and Tamarindus, which have also demonstrated anthelmintic properties, underscoring the value of plant-derived compounds in helminth control [22]. Further exploration is warranted to determine whether these compounds work synergistically or independently, as understanding their interactions could enhance the development of effective anthelmintic therapies.

The anthelmintic activity of *D. longan* extract is likely due to the presence of tannins and phenolic compounds, which bind to proteins and disrupt energy generation, leading to paralysis and death in helminth parasites [25]. For example, alkaloids present in the extract may interfere with neuromuscular transmission in helminths, potentially causing paralysis by disrupting the release or action of neurotransmitters [33]. Flavonoids might contribute to anthelmintic activity by enhancing the permeability of the worm's cuticle, making it more susceptible to the effects of other bioactive compounds in the extract [34]. Additionally, saponins are known to form complexes with cholesterol in cell membranes, which could compromise the integrity of the helminths' cellular structures, leading to their death [35]. These results suggest that *D. longan* could serve as a natural treatment option, potentially as an alternative or complementary approach to conventional anthelmintic drugs. However, further research is needed to determine the optimal dosage, assess potential side effects, and understand the underlying mechanisms.

In conclusion, *D. longan* extract has shown significant anthelmintic activity against tapeworms, indicating its potential as a natural remedy for helminth infections. The findings suggest that *D. longan* could be particularly valuable in regions where conventional treatments, such as piperazine, are less accessible or where issues of resistance limit their effectiveness. Further research is required to fully evaluate its therapeutic potential, optimize its use, and develop standardized formulations for clinical application.

REFERENCES

1. Bauri RK, Tigga MN, Kullu SS. A review on use of medicinal plants to control parasites. Indian J Nat Prod Resour. 2015;6(4):268-77.

2. Mukherjee M, Stoddart A, Gupta RP, Nwaru BI, Farr A, Heaven M, et al. The epidemiology, healthcare and societal burden and costs of asthma in the UK and its member nations: analyses of standalone and linked national databases. BMC Med. 2016;14:113.

3. Asiimwe E, Ondari EN, Odongo GA, Kimanje RK. Determination of phytochemicals and anthelmintic activity of Rytigynia kigeziensis Verdc extracts using Eudrilus eugeniae model. World J Adv Res Rev. 2023;17(03):147-56. doi:10.30574/wjarr.2023.17.3.0320.

4. Wright JE, Werkman M, Dunn JC, Anderson RM. Current epidemiological evidence for predisposition to high or low intensity human helminth infection: a systematic review. Parasit Vectors. 2018 Jan 31;11(1):65. doi: 10.1186/s13071-018-2656-4.

5. Njambi E, Magu D, Masaku J, Okoyo C, Njenga SM. Prevalence of intestinal parasitic infections and associated water, sanitation, and hygiene risk factors among school children in Mwea Irrigation Scheme, Kirinyaga County, Kenya. J Trop Med. 2020 May 11;2020:3974156. doi: 10.1155/2020/3974156.

6. Kungu JM, Dione MM, Ejobi F, Harrison LJ, Poole EJ, Pezo D, Grace D. Sero-prevalence of Taenia spp. cysticercosis in rural and urban smallholder pig production settings in Uganda. Acta Trop. 2017 Jan;165:110-5. doi: 10.1016/j.actatropica.2016.01.016.

7. Rahantamalala A, Rakotoarison RL, Rakotomalala E, Rakotondrazaka M, Kiernan J, Castle PM, et al. Prevalence and factors associated with human Taenia solium taeniosis and cysticercosis in twelve remote villages of Ranomafana rainforest, Madagascar. PLoS Negl Trop Dis. 2022;16(4). doi:10.1371/journal.pntd.0010265.

8. Lesh EJ, Brady MF. Tapeworm. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan-. [updated 2023 Aug 28; cited 2024 Aug 14]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK537154/.

9. Gulelat Y, Eguale T, Kebede N, Aleme H, Fèvre EM, Cook EAJ. Epidemiology of porcine cysticercosis in eastern and southern Africa: systematic review and meta-analysis. Front Public Health. 2022 Mar 16;10:836177. doi: 10.3389/fpubh.2022.836177.

10. Alarakol SP, Bagaya BS, Onen-Yagos W, Odongo-Aginya EI. Prevalence and risk factor associated with Taenia solium cysticercosis among pig farmers in two districts (Amuru and Gulu) in Northern Uganda. J Parasitol Vector Biol. 2021;13(1):25-34. doi:10.5897/JPVB2020.0395.

11. Adriko M, Tinkitina B, Tukahebwa EM, Standley CJ, Stothard JR, Kabatereine NB. Data on the pre-MDA and post-MDA interventions for Schistosoma mansoni and Schistosoma haematobium in a co-endemic focus in Uganda: 1951-2011. Data Brief. 2018 Sep 6;20:991-8. doi: 10.1016/j.dib.2018.08.200.

12. Degarege A, Erko B, Negash Y, Animut A. Intestinal helminth infection, anemia, undernutrition and academic performance among school children in northwestern Ethiopia. Microorganisms. 2022 Jul 5;10(7):1353. doi: 10.3390/microorganisms10071353.

13. Joshi UP, Wagh RD. In vitro anthelmintic activity of Maytenus emarginata stem bark on Indian adult earthworm. Asian J Pharm Clin Res. 2019;12(3):400-3. doi:10.22159/ajpcr.2019.v12i3.30756.

14. Aydın Ç, Mammadov R. Anthelmintic activities of methanol extracts of five different geophyte plant species. Kahramanmaraş Sütçü İmam Üniversitesi Tarım Ve Doğa Dergisi. 2022;25(Ek Sayı 2):376-81. doi:10.18016/ksutarimdoga.vi.1179200.

15. Paul P, Biswas P, Dey D, Saikat ASM, Islam MA, Sohel M, et al. Exhaustive plant profile of Dimocarpus longan Lour with significant phytomedicinal properties: a literature-based review. Processes. 2021;9(10):1803. doi:10.3390/pr9101803.

16. Rakariyatham K, Zhou D, Rakariyatham N, Shahidi F. Sapindaceae (Dimocarpus longan and Nephelium lappaceum) seed and peel by-products: potential sources for phenolic compounds and use as functional ingredients in food and health applications. J Funct Foods. 2020;67:103846. doi:10.1016/j.jff.2020.103846.

17. Natthakarn C, Supanimit T, Ampai P, Nirush L. Acute and chronic oral toxicity assessment of longan sugar extracts derived from whole fruit and from fruit pulp in rats. J Ethnopharmacol.

2020;263:113184 https://doi.org/10.1016/j.jep.2020.113184.

18. Bruna T, Maldonado-Bravo F, Jara P, Caro N. Silver nanoparticles and their antibacterial applications. Int J Mol Sci. 2021;22(13):7202. doi:10.3390/ijms22137202.

19. Shekhawat N, Vijayvergia R. Anthelmintic activity of extracts of some medicinal plants. Int J Comput Sci Math. 2011;3(2):183-7.

20. Chandrashekhar CH, Latha KP, Vagdevi HM, Vaidya VP. Anthelmintic activity of the crude extracts of Ficus racemosa. J Chem. 2008;2(2):2-5.

21. 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.

22. Ishnava KB, Konar PS. In vitro anthelmintic activity and phytochemical characterization of Corallocarpus epigaeus (Rottler) Hook. f. tuber from ethyl acetate extracts. J Ethnopharmacol. 2020;1-10.

23. Hishamuddin SN, Amir QQ, Mohamed AH, Said NR. Extraction of bioactive compounds from longan peel by using solvent extraction method and its antioxidant activity. ASM Sci J. 2020;13(6):60-5.

24. Nakitto S, Odoma S, Enyinna O, Usman IM. Prophylactic anti-ulcer effect of aqueous leaf extract of Mormodica foetida (Schumach & Thonn) against indomethacin-induced ulcers in Wistar rats. Trop J Nat Prod Res. 2023;7(11):5231-5. doi:10.26538/tjnpr/v7i11.30.

25. Aderibigbe SA, Idowu SO. Anthelmintic activity of Ocimum gratissimum and Cymbopogon citratus leaf extracts against Haemonchus placei adult worm. Afr J Tradit Complement Altern Med. 2020;17(1):8-12.

26. Bereda G. Anthelmintic agents: vermicide and vermifuge. Insights Biol Med. 2022;6:1-8.

27. Wang M, Chen T, Wang Q, Shi Y. Antioxidant, bacteriostatic and preservative effects of extractable condensed tannins isolated from longan pericarps and seeds. Plants. 2023;12(3). doi:10.3390/plants12030512.

28. Depeursinge A, Racoceanu D, Iavindrasana J, Cohen G, Platon A, Poletti PA, Muller H. Fusing visual and clinical information for lung tissue classification in HRCT data. Artif Intell Med. 2010;83. doi:10.1016/j.artmed.2010.05.001.

29. Parag S, Katiyar C, Katiyar S. Anti-cancer and anti-aging activities of Dimocarpus longan Lour extracts. J Ethnopharmacol. 2010;132(2):338-44.

30. Huang GJ, Wang BS, Lin WC, Huang SS, Lee CY, Yen MT, et al. Antioxidant and anti-inflammatory properties of longan (Dimocarpus longan Lour.) pericarp. Evid Based Complement Alternat Med. 2012;2012:709483. doi:10.1155/2012/709483.

31. Sillanpää M, Engström MT, Tähtinen P, Green RJ, Käpylä J, Näreaho A, Karonen M. Tannins Can Have Direct Interactions with Anthelmintics: Investigations by Isothermal Titration Calorimetry. Molecules. 2023; 28(13):5261. https://doi.org/10.3390/molecules28135261

32. Lima CS, Pereira MH, Gainza YA, Hoste H, Regasini LO, Chagas AC. Anthelmintic effect of Pterogyne nitens (Fabaceae) on eggs and larvae of Haemonchus contortus: Analyses of structure-activity relationships based on phenolic compounds. Ind Crops Prod. 2021;164:113348. doi:10.1016/j.indcrop.2021.113348.

33. Adak M, Kumar P. Herbal anthelmintic agents: a narrative review. J Tradit Chin Med. 2022;42(4):641-651. doi: 10.19852/j.cnki.jtcm.2022.04.007.

34. Mordvinov VA, Ponomarev DV, Pakharukov YV, Pakharukova MY. Anthelmintic Activity of Antioxidants: In Vitro Effects on the Liver Fluke Opisthorchis felineus. Pathogens. 2021,2;10(3):284. doi: 10.3390/pathogens10030284.

35. Maestrini M, Tava A, Mancini S, Salari F, Perrucci S. In Vitro Anthelmintic Activity of Saponins Derived from Medicago spp. Plants against Donkey Gastrointestinal Nematodes. Vet Sci. 2019;29;6(2):35. doi: 10.3390/vetsci6020035.