

# Anthelmintic Effects of *Marsdenia tenacissima* Root Extracts on *Pheretima posthuma*: Role of Extraction Method and Phytochemistry

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**Abstract** The study investigates the extraction methods, phytochemical profile, and anthelmintic activity of alcoholic (A) and hydroalcoholic (HA) of *Marsdenia tenacissima* (MT) root. Using maceration (M) and Soxhlet extraction (S), four extracts were prepared namely, MTAM, MTHAM, MTAS, and MTHAS with yields of 8.42%, 9.75%, 12.97%, and 11.25%, respectively. Soxhlet extraction produced higher yields overall, particularly with MTAS, due to its continuous solvent circulation. The choice of solvent also influenced yields, with hydroalcoholic mixtures performing better in extracting bioactive compounds. GC-MS analysis identified a total of 423 compounds across the extracts, with MTAS containing 114, MTHAS 101, MTAM 101, and MTHAM 107 compounds. Notably, MTAS and MTHAS, as well as MTAM and MTHAM, each shared 26 compounds, indicating overlapping phytochemical compositions among the extracts. The soxhlet and maceration extractions shared 58 similar compounds. The anthelmintic activity of these extracts was identified using the adult Indian earthworm, *Pheretima posthuma*, with paralysis and death times recorded. Albendazole, used as a positive control, exhibited significant activity, with a dose-dependent reduction in paralysis and death times. Among the *M. tenacissima* extracts, MTAS at 50 mg/mL demonstrated the shortest paralysis ( $79.6 \pm 3.12$  minutes) and death times ( $125.4 \pm 2.48$  minutes), highlighting its potential anthelmintic efficacy. These findings suggest that

*Marsdenia tenacissima*, particularly the MTAS extract, possesses promising anthelmintic properties. Soxhlet extraction and the use of alcoholic solvents enhance the yield of bioactive compounds and the overall anthelmintic effect, supporting further exploration of this plant as a potential source of anthelmintic agents.

**Keywords** *Marsdenia tenacissima*, Soxhlet, Maceration, Anthelmintic, *Pheretima posthuma*

## 1. Introduction

Helminthic infections caused by parasitic worms, such as nematodes, trematodes, and cestodes, rank among the most common neglected tropical diseases in light of over 1.5 billion infected worldwide or around 24% of the world's population [1]. They thrive in deprived regions due to a lack of hygiene and sanitation facilities and they can subsist on soil, the infections being passed through the consumption of contaminated food and water [2]. Together, these helminths- *Ascaris lumbricoides*, *Trichuris trichiura*, and hookworms-infect more than 820 million children and over 450 active cases, causing significant burdens to health [3]. Helminthic infections are often chronic and not immediately lethal, yet they contribute annually to about 135 000 deaths, either through direct effects of

complications arising from anemia, malnutrition, and immune suppression. These infections go a long way in causing a host of health problems, like retardation in the cognitive abilities of children, poor academic performance, and low productivity in adults, thereby perpetuating cycles of poverty and disease [4]. Mass Drug Administration (MDA) has therefore remained the mainstay in attempts at combating these widespread infections through the use of synthetic anthelmintics, including Albendazole [5] and mebendazole [6], as well as ivermectin [7], among target populations. Some areas have already been successful with MDA in reducing the infection rate, but a number of other challenges already threaten long-term control efforts, including high reinfection rates and increasing drug resistance [8, 9]. Other safety issues involve some of the synthetic anthelmintics with certain adverse effects in groups such as children and pregnant women. Such a scenario has created a need for safer, sustainable alternatives [10].

Natural products have historically served as the basis for many modern pharmaceuticals, and plants with medicinal properties are often explored for their pharmacological potential. Plants produce vast quantities of secondary metabolites, including alkaloids, flavonoids, saponins, and tannins that are essential for various biological activities, and among these include anthelmintic effects. These bioactive agents interfered with critical pathways in parasitic worms by disrupting cell membrane integrity, energy metabolism, ion transport, and neurotransmitter regulation that ultimately could be lethal for the helminths, causing paralysis [11, 12]. In recent years, *Marsdenia tenacissima* from the Apocynaceae family, has garnered attention in traditional and modern medicine for its wide-ranging therapeutic properties. *M. tenacissima* is widespread throughout India, Southeast Asia, and China. Tenacissima has been used in Ayurveda and Traditional Chinese Medicine (TCM) practices for thousands of years [13]. The roots have been used due to their anti-inflammatory [14], anti-tumor [15], immunomodulatory [16], and hepatoprotective [17] activities. Phytochemical studies have established the presence of steroidal saponins, flavonoids, glycosides, and alkaloids in *M. tenacissima* and it is believed to be responsible for its pharmacological effects [18, 19].

Although it is very rich in phytochemicals and has extensive medicinal use, it has not been studied carefully in the scientific literature. In this backdrop, the anthelmintic potential of *M. tenacissima* has been assayed. Given the increasing interest in identifying alternative anthelmintic agents from natural sources, this study aims to evaluate the anthelmintic efficacy of *M. tenacissima* root extracts against the model organism *Pheretima posthuma*, commonly known as the Indian earthworm. *Pheretima posthuma* is a very viable model organism in the anthelmintic study due to its similarities with the human parasitic worms in terms of physiological and anatomical features [20]. The nervous system of earthworms, like that

of parasitic nematodes, operates primarily through cholinergic transmission, making them susceptible to compounds that target neuromuscular systems in a manner comparable to the effects seen in helminths. As such, the earthworm model is relevant for direct observation of paralysis and death as indicators of anthelmintic activity, and thus potentially reliable sources of information on the efficacy and even mechanism of action of test compounds. Considering the bioactive compounds in *M. tenacissima* roots, which are alkaloids, terpenoids, saponins, steroids, esters, lipids, organic acids, etc., the extracts might exhibit anthelmintic effects on *P. posthuma* and would justify its traditional application as an anti-parasitic drug. We have also compared the effectiveness of the roots of *M. tenacissima* extracted by different extraction methods and against a standard anthelmintic drug to develop a basis for potential applications in both traditional and modern medicine.

## 2. Materials and Methods

### 2.1. Collection, Authentication and Preparation of Root Extracts

The roots of *M. tenacissima* were collected from Tirunelveli and were authenticated by Dr. S. Mutheswaran, Xavier Research Foundation, Tamilnadu (XCH-40420). Cleaned rootstock of *M. tenacissima* was dried at room temperature at a shaded area and crushed into fine powder for maceration extraction. Two extracts, namely, alcoholic macerate (MTAM) and hydroalcoholic macerate (MTHAM) were prepared separately by mixing 100g of root powder with 100% alcohol for MTAM and aqueous alcohol solution (80:20) for MTHAM. The mixtures were left to macerate in sealed glass containers at room temperature for 72 hours, with the periodic shaking. Then the mixtures were filtered, and the filtrates were concentrated using rotary evaporator at low temperature until the semi-solid extracts were obtained and labeled as MTAM and MTHAM, and stored in airtight containers [21]. Another set of powder was once more divided for Soxhlet extraction into two, alcoholic Soxhlet extract (MTAS), and hydroalcoholic Soxhlet extract (MTHAS). The powdered roots were extracted using 100% alcohol for MTAS, and aqueous alcohol solution (80:20) for MTHAS, in a Soxhlet apparatus. The solvent was extracted for 8 hours for effective cycling of the solvent. The extracts were cooled and filtered to produce semi-solid forms. These extracts were labelled as MTAS and MTHAS, later stored in airtight containers for further use [22].

### 2.2. GC-MS Analysis

The extracts were subjected to analysis by GC-MS on a GC-2010 instrument at a column oven temperature of 50.0°C. The injection temperature was set to 250.0°C, and

the injections were carried out in split mode. Flow control was maintained in pressure mode, regulated at 53.5 kPa. The column flow rate was held constant at 1.00 mL/min and the overall flow rate was maintained at 54.0 mL/min. The linear velocity of the carrier gas was set at 36.3 cm/sec. The purge flow utilized was at 3.0 mL/min with a split ratio of 50.0 for proper introduction of samples with minimum possibilities of sample overload in the column. These conditions were consistently applied across all extract samples to enable accurate identification and quantification of the compounds present [23].

### 2.3. Anthelmintic Activity

Anthelmintic activity of *M. tenacissima* extracts was determined on the adult Indian earthworm *Pheretima posthuma* with the method suggested by Subash et al. [24]. Standard reference drug albendazole was dissolved in normal saline to get concentrations of 25 mg/mL and 50 mg/mL. Transferred to various Petri dishes, these were used for the experiment. Alcoholic and hydroalcoholic extracts of *M. tenacissima* solution, were diluted with normal saline up to concentrations of 25 mg/mL and 50 mg/mL. Normal saline (0.9% NaCl) alone is prepared as a normal control. Each solution was added to the prepared Petri dishes. Five groups of earthworms (n = 5), similar in length, around 8–10 cm were selected and placed into the prepared Petri dishes at room temperature. The paralysis time was measured at which the worms were completely static regardless of vigorous shaking. After verifying the worms to be static, they were left shaken or soaked in warm water at 50°C to measure the death time. The minutes taken both for paralysis and death for all the sample groups were recorded.

## 3. Results

### 3.1. Extraction of *M. tenacissima* Roots

The maceration and soxhlet extraction processes yielded four different extracts, and the percentage yields of these extracts were 8.42% for MTAM, 9.75% for MTHAM, 12.97% for MTAS, and 11.25% for MTHAS. Higher

percentage yields have been reported through soxhlet extraction when compared with maceration, with the MTAS extract having the highest yield at 12.97%, followed by MTHAS at 11.25%. The choice of solvent also influenced the yield. The slightly higher yield in the hydroalcoholic macerate (MTHAM, 9.75%) compared to the alcoholic macerate (MTAM, 8.42%) aligns with findings, where the addition of water to alcohol improves the extraction of various bioactive compounds. The hydroalcoholic solvent system gave an extraction of 11.25% by soxhlet which was lower than that obtained by MTAS but significantly higher than the macerates, indicating that although Soxhlet extraction tends to enhance the overall yield, polarity of the solvent greatly influences the efficiency of extraction of compounds. These can explain the higher yield that this Soxhlet method can obtain in a process because of the continuous circulation of fresh solvent through the plant material, ensuring better extraction efficiency of phytochemicals than in maceration

### 3.2. Molecular Weight and Formula Determination by GCMS Analysis

GC-MS of the four samples MTAS (Figure 1), MTHAS (Figure 2), MTAM (Figure 3), and MTHAM (Figure 4) showed a very broad and varied profile of bioactive compounds where each sample had a unique chemical fingerprint. In total, there were 423 compounds identified from the four samples put together. Qualitatively, there were 114 compounds in MTAS, 101 compounds in MTHAS, 101 compounds in MTAM, and 107 compounds in MTHAM. Interestingly, some of the compounds were common to some pairs of samples, which would point out to the similarity in the phytochemical compositions of these samples. Overlap as high as 26 compounds common to the MTAS and MTHAS samples could suggest a core set of bioactive constituents of the two samples, perhaps pointing to a close origin or extraction of the chemical. Additionally, MTAM and MTHAM also shared 26 compounds, highlighting another subset of consistent constituents within this pair. Likewise, the soxhlet extraction and the maceration extraction shared 58 similar compounds (Figure 5).



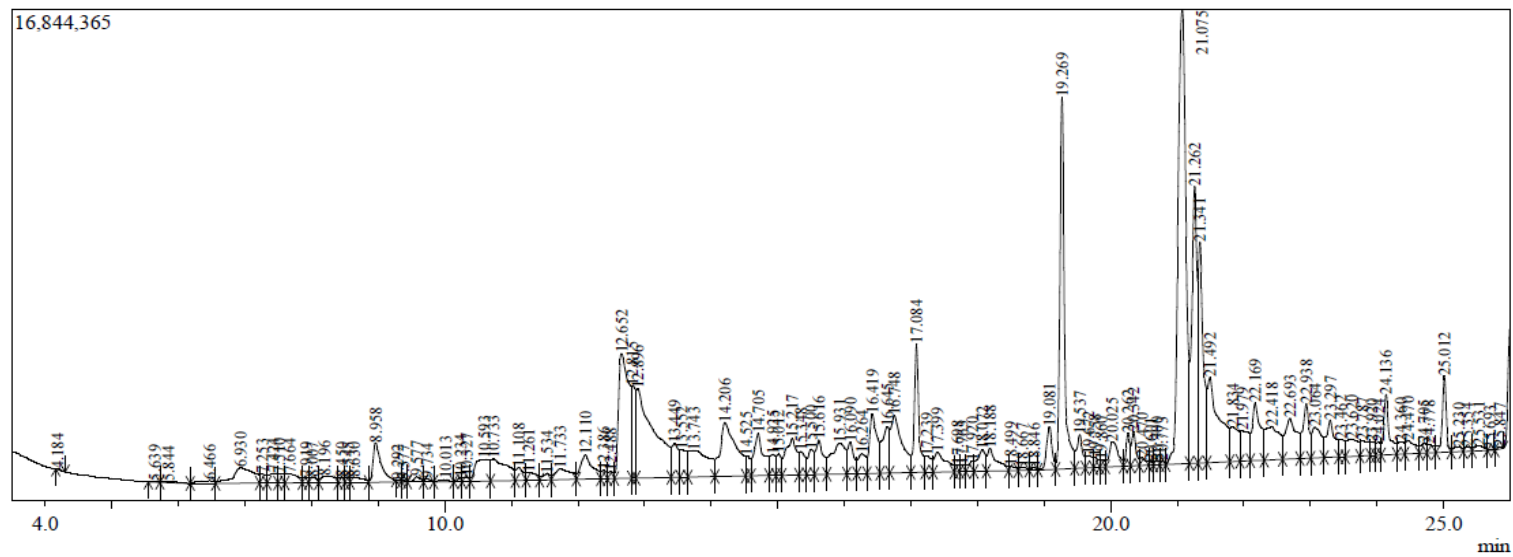


Figure 3. GCMS analysis of *M. tenacissima* alcohol maceration extraction

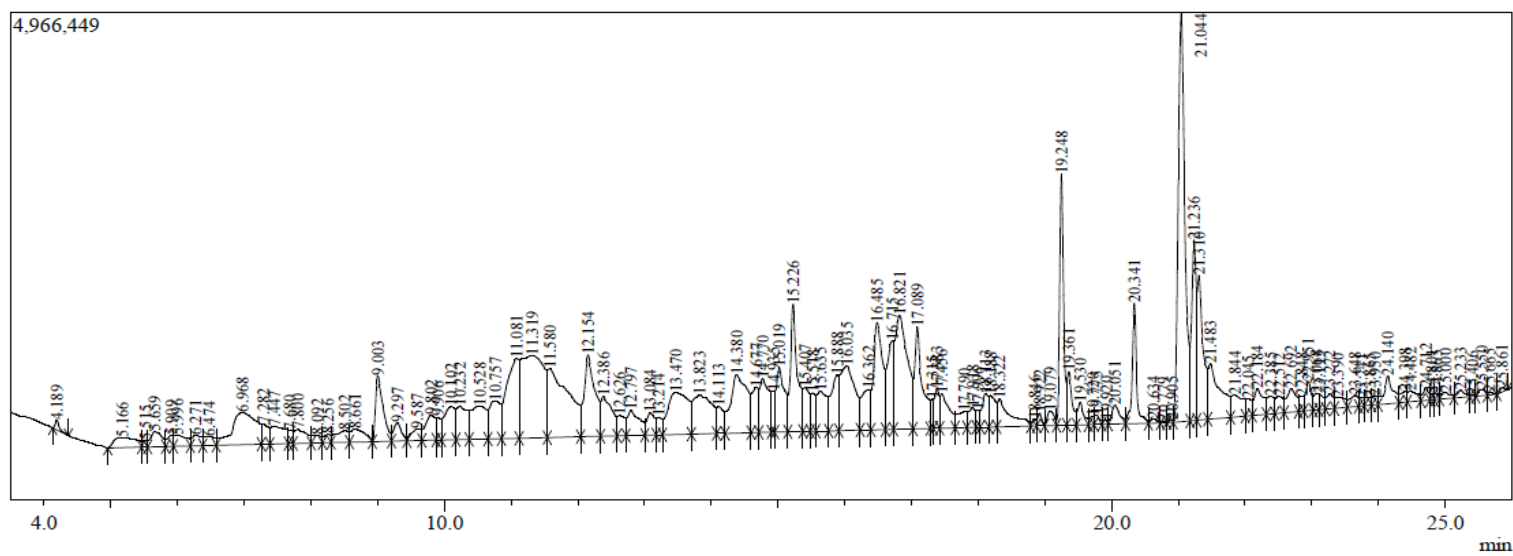


Figure 4. GCMS analysis of *M. tenacissima* hydroalcohol maceration extraction

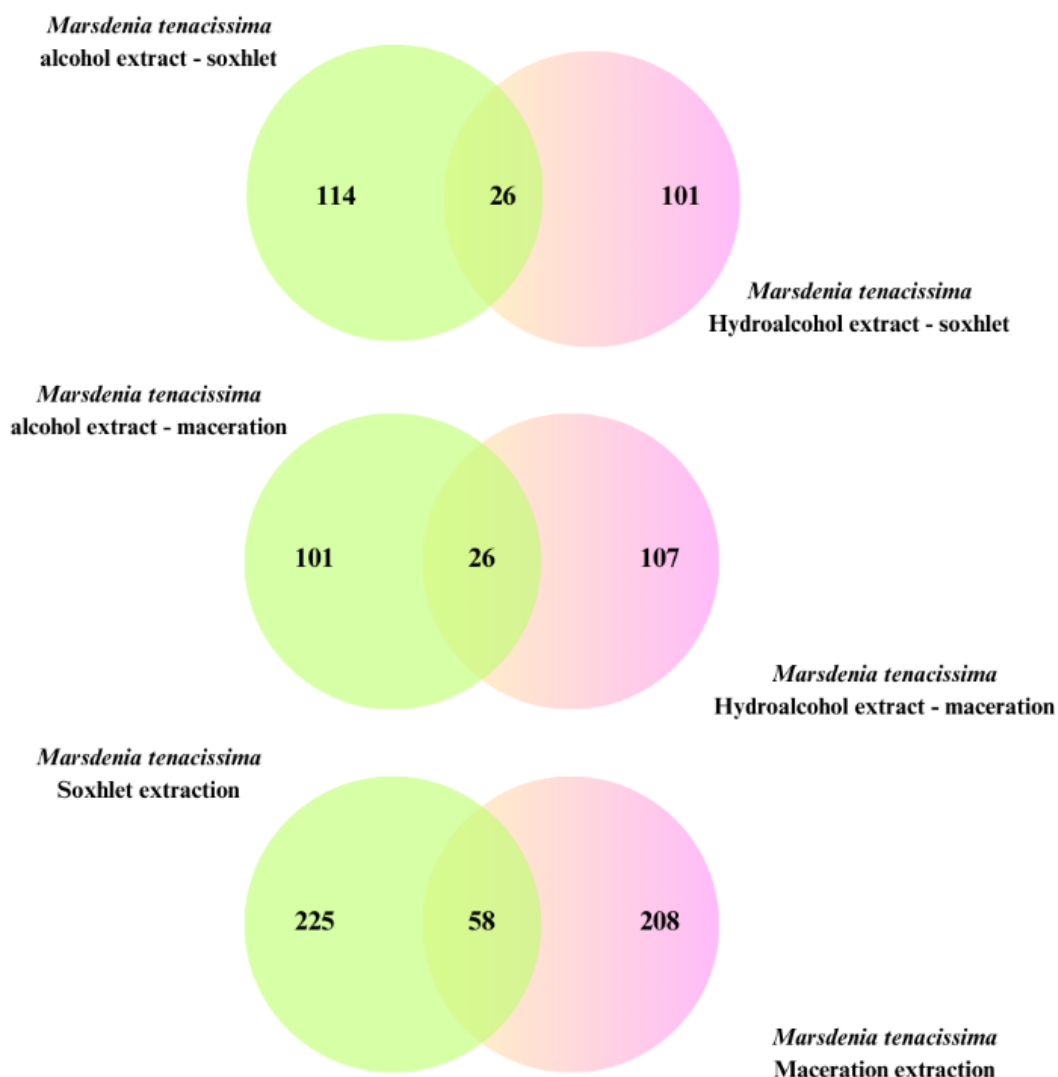


Figure 5. Compound identified in the *M. tenacissima* extracts

The diversity of compounds present in the MTAS extract was mainly terpenoids, fatty acids, phenolics, esters, and alcohols. It was dominated by terpenes like neophytadiene, ar-turmerone, and (-)-(1S,2R,4R)-beta-fenchol, which explain the strong volatile and aromatic character of the extract. Fatty acids and esters may also be well predicted as they are available in good amounts, since n-decanoic acid and dodecanoic acid are found regularly in lipid-rich extracts. The phenolic content in MTAS, represented by 2-methoxy-phenol, 2-methoxy-4-vinylphenol and 1,2-benzenediol, may also contribute to the pharmacological activity. Other alcohol derivatives such as glycerol and other long-chain alcohols were also detected, so making the MTAS extract more biologically complex. The MTHAS extract constituted 101 compounds, which itself represented a rich phytochemical profile by having terpenoids, fatty acids, phenolics, esters, and alkaloids as the major classes. Terpenoids were highly abundant, thus largely responsible for the aromatic and potentially medicinal nature of the extract; notable compounds in this

category included 1-hexen-3-ol and 2-methoxy-4-vinylphenol.

This extract had a total of 101 compounds characterized by high abundance of fatty acids, alcohols, terpenoids, and phenolics. Fatty acids were the most abundant compounds in this extract; they included key components such as octadecanoic acid, linoelaidic acid, tetradecanoic acid, and heptanoic acid that give evidence of a high lipophilic content. Alcohols such as 1-octadecanol and cyclohexanol were also significant, and both the volatility and solubility aspects of the extract are attributed to them. Terpenoids and their derivatives like ar-turmerone and cis-sabinene were also highly concentrated that leads to the enhanced aromatic and bioactive profile for MTAM. The extract for MTHAM consists of 107 compounds with a well-balanced fatty acids profile of lactones, esters, alcohols, and phenolics. The incidences of fatty acids like cis-10-heptadecenoic acid, dodecanoic acid, tetradecanoic acid, and n-decanoic acid were higher, which reflects the lipid richness of this extract. There were lactones present, and

among these was pantolactone. The phenolic compounds identified in this extract were consistent with those observed in other extracts. These common molecules might explain all the apparent biological activities observed between the extracts; they could explain any synergy in the therapeutic activity. Profiling of individual compound

mixtures shows distinct profiles for each group. This means that the functional capacity as well as potential applications is inferred to be different for each sample. The findings provide a foundation for identifying novel compounds of interest within these samples, particularly those that are unique or have low overlap with other samples.



**Figure 6.** Anthelmintic activity of *M. tenacissima* extracts and Albendazole. A-normal control; B-abendazole (25 mg/mL); C-abendazole (50 mg/mL); D-MTAS (25 mg/mL); E-MTAS (50 mg/mL); F-MTHAS (25 mg/mL); G-MTHAS (50 mg/mL); H-MTAM (25 mg/mL); I-MTAM (50mg/mL); J-MTHAM (25 mg/mL); K-MTHAM (50 mg/mL)

### 3.3. Anthelmintic Activity

The anthelmintic activity of the *M. tenacissima* extracts was studied by measuring paralysis and death times in adult Indian earthworm, *Pheretima posthuma* (Figure 6). The values are presented as means  $\pm$  SD,  $n = 5$  in each set. With the normal control group, paralysis and death were zero for both stages. This serves as a baseline for comparison, indicating that without any treatment, the subjects remain unaffected. In comparison, albendazole at 25 mg/mL and 50 mg/mL concentrations, used as a positive control, showed significant anthelmintic activity, with a paralysis time of  $55 \pm 2.4$  minutes and  $42.6 \pm 2.08$  minutes, respectively (Figure 6 & Figure 7). The death times for albendazole at 25 mg/mL and 50 mg/mL were recorded as  $135.4 \pm 2.32$  minutes and  $123 \pm 2$  minutes, respectively, demonstrating a dose-dependent increase in efficacy. Among the *M. tenacissima* extracts, MTAM at 25 mg/mL and 50 mg/mL concentrations produced paralysis times of  $114.2 \pm 3.84$  and  $94 \pm 3.2$  minutes, and death times of  $195.8 \pm 3.04$  and  $188.8 \pm 3.84$  minutes, respectively (Figure 7 & Figure 8).

The MTHAM demonstrated a longer paralysis and death time, with  $217.2 \pm 4.16$  and  $191.6 \pm 4.48$  minutes for paralysis, and  $267.2 \pm 2.64$  and  $242 \pm 3.2$  minutes for death, at 25 mg/mL and 50 mg/mL, respectively. The MTAS exhibited paralysis times of  $115.6 \pm 4.08$  and  $79.6 \pm 3.12$  minutes, and death times of  $196.4 \pm 2.88$  and  $125.4 \pm 2.48$  minutes at 25 mg/mL and 50 mg/mL concentrations, respectively. Lastly, the MTHAS showed paralysis times of  $148.2 \pm 2.96$  and  $118.8 \pm 3.84$  minutes, and death times of  $215.4 \pm 4.32$  and  $184.6 \pm 4.08$  minutes at 25 mg/mL and 50 mg/mL concentrations, respectively (Figure 7 & Figure 8).

Overall, the anthelmintic activity of Albendazole was the strongest since it caused paralysis and death times that were significantly shorter than those of all the *M. tenacissima* extracts. Among the extracts, relatively the better activities were shown by the alcoholic and hydroalcoholic Soxhlet extracts MTAS and MTHAS at 50 mg/mL concentration. They showed the lowest paralysis and death times but were less potent compared to the standard drug, albendazole.

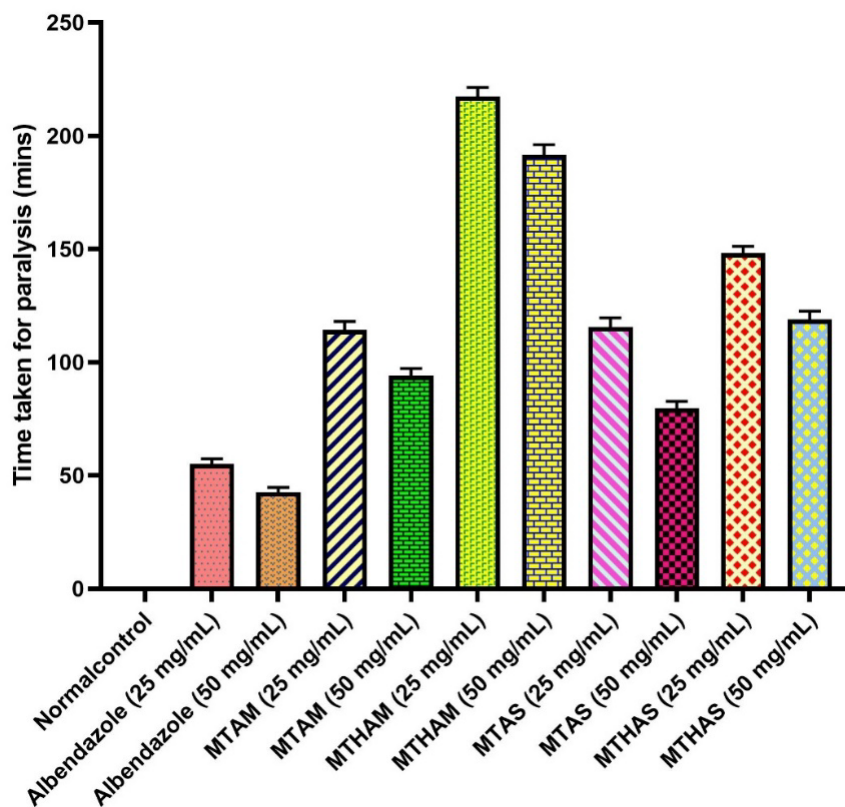


Figure 7. Time taken for paralysis

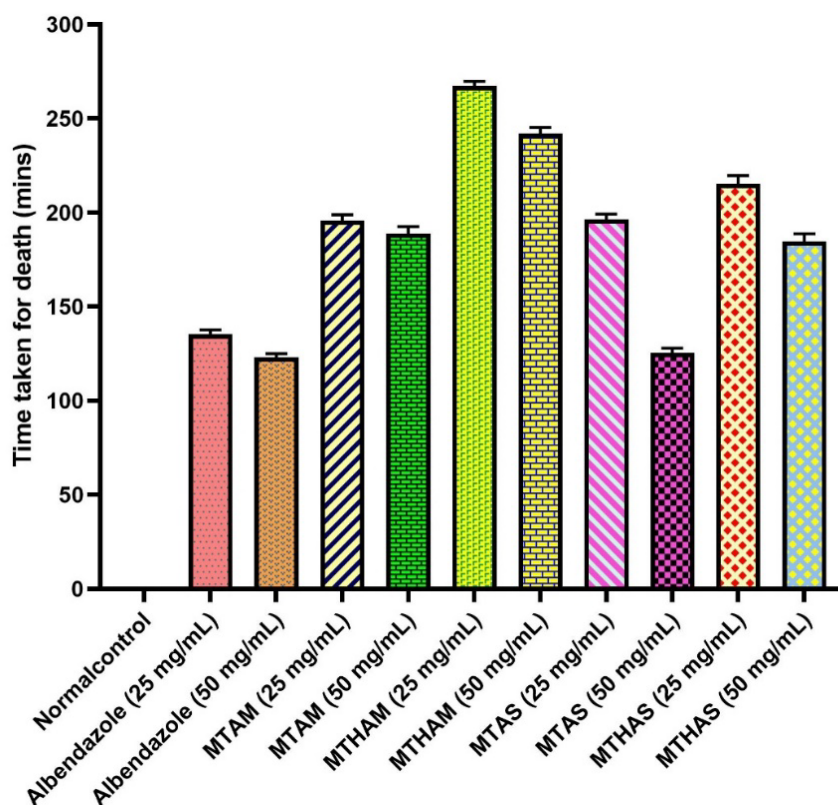


Figure 8. Time taken for death

## 4. Discussion

Traditionally, the roots of *Marsdenia tenacissima* are used in folk medicine and Chinese medicine as an anthelmintic. However, scientific evidence to support this is limited. Thus, the present study was made to determine the anthelmintic activity of *M. tenacissima* and to supply a scientific basis for its traditional deworming practice. Anthelmintic activity demonstrated that the extracts were effective in the order of MTAS>MTHAS>MTAM>MTHAM. These results would suggest that the extraction method combined with the type of solvent may indeed play an important role in determining bioactivity for plant extracts, which is consistent with many studies related to the extraction of bioactive plant compounds [25-27].

Soxhlet extraction is known for its ability to continuously recycle the solvent, which maximizes the extraction of bioactive compounds from plant material [28]. Soxhlet extraction in this work resulted in a very high yield of compounds, as implied by the number of compounds isolated in MTAS (114 compounds) and MTHAS (101 compounds) in comparison to maceration extracts MTAM with 101 compounds and MTHAM with 107 compounds. This brings out the efficiency of Soxhlet extraction, since material from the plant is always in contact with the solvent for a reasonable time, thus more effective extraction of both polar and non-polar constituents. Maceration is simpler and

less energy-consuming compared to Soxhlet; however, it is not very effective, mainly for compounds that do not dissolve well in the solvent used or those which take up a longer period for better recovery [29]. The relatively lower number of compounds found in the macerated extracts (MTAM and MTHAM) likely reflects the lack or lower concentration of the phytochemicals during the extraction process, which directly correlates with the observed lower anthelmintic activity.

The polarity of a solvent greatly affects the efficiency of extraction and the composition of bioactive compounds in the process [30]. In this study, alcoholic and hydroalcoholic solvents were used in the preparation of the extracts; alcohol was preferred for MTAS and MTAM, whereas an 80:20 ratio of alcohol to water was utilized for MTHAS and MTHAM. Alcohol is known to be a versatile solvent capable of extracting a wide range of bioactive compounds, including alkaloids, flavonoids, and terpenoids, all of which have been associated with anthelmintic properties [31]. To great surprise, the extracts obtained by Soxhlet with alcohol as the solvent had the highest potency of anthelmintic activity followed by the hydroalcoholic Soxhlet extract. This would indicate that though hydroalcoholic mixtures can extract a wider spectrum of compounds, the higher efficiency of alcohol in solvent extraction using soxhlet apparatus may be the reason for better biological activities in MTAS. Alcohol is one such solvent that tends to dissolve semi-polar as well as non-

polar compounds quite efficaciously [30, 32]. This may be the reason why the anthelmintic activity-involved active constituents proved more potent in the case of MTAS, wherein they seem more potent than in MTHAS. Although hydroalcoholic solvents are preferred for extraction of a broader range of compounds, added water in MTHAS may already have suppressed the solubility of some active constituents, thus diluted their concentration and worked out to be less bioactive compared to alcohol alone.

GC-MS profile of root extract showed various bioactive compounds such as phenol, 5-methyl-2-(1-methylethyl)-, phenol, 3,5-bis(1,1-dimethylethyl)-, n-hexadecanoic acid, heptadecanoic acid, oleic acid and octadecanoic acid. These have also been reported to be active as anthelmintic in several studies; either in vitro or synergistically in extracts from plants. Each of these compounds possesses some very unique biochemical properties that qualify them to be pretty potent anthelmintic agents in their own right, particularly against the parasitic helminths *Haemonchus contortus*, *Schistosoma species*, and *Strongyloides species*. Phenol, 5-methyl-2-(1-methylethyl)- has shown significant in vitro anthelmintic activity against *Haemonchus contortus* as highlighted in studies of methanolic plant extracts from Kenya, where it likely acts by disrupting cellular functions within the parasite, leading to mortality [33]. Similarly, phenol, 3,5-bis(1,1-dimethylethyl)- has been isolated and screened in *Plectranthus mollis Spreng* for its potent anthelmintic activity, supporting its role in disrupting parasite survival mechanisms [34]. The phenolic structure of these compounds may contribute to their mode of action, involving oxidative stress induction or interference with cellular signaling pathways essential for parasite viability.

n-hexadecanoic acid (palmitic acid) and octadecanoic acid (stearic acid) are saturated fatty acids recorded as anthelmintic, particularly from the extracts of *Corallocarpus epigaeus* tuber [35]. These fatty acids may exert their effects by changing the lipid content composition of parasitic cell membranes thereby lowering their stability and eventually killing them through cellular disintegration. This mechanism opens up the possibility that these fatty acids are drugs for compromising survival through structural vulnerability. A lesser-explored saturated fatty acid, heptadecanoic acid, also has been found with anthelmintic activity in metabolic profiling and bioactivity assays much like that identified in *Picria felterrae* Lour [36]. Its anthelmintic activity has been suggested to be related to its capacity for binding and disrupting parasite membranes, although further research is required to determine its molecular targets. The presence of heptadecanoic acid in this root extract adds value to its bioactive profile, broadening the spectrum of potential anthelmintic efficacy. Oleic acid, a monounsaturated fatty acid, has shown remarkable antischistosomal activity, as demonstrated by studies utilizing oleic acid-loaded polymeric nanocapsules for controlled oral administration [37]. The compound has already been known to cause cell

lysis of *Schistosoma* parasites through disruption of their cellular membranes. Bioactivity of oleic acid in controlling the parasites obviously is therapeutically important, whether as an active drug itself or as part of a composition where its bioavailability has been enhanced.

Collectively, these six compounds present a multi-targeted approach to anthelmintic activity. The presence in the root extract opens synergistic potential where each constituent might complement other constituents that show efficacy because of increased effectiveness when used against helminthic infections. Thus, the identified anthelmintic activity of the root extract may be ascribed to a concerted action of these bioactive molecules, making the MTAS extract a promising natural source for anthelmintic therapy. Further in-depth studies on their modes of action, particularly in vivo, could yield valuable insights into the development of natural anthelmintic formulations.

## 5. Limitations

The study has certain limitations, especially in the choice of solvents, drying, and extraction conditions. Firstly, the limitation of hydroalcoholic and alcoholic solvents may not be an accurate reflection of the overall phytochemical content of the plant material, as non-aqueous solvents such as chloroform or hexane would be more effective in the extraction of certain compounds. Solvent polarity has a strong impact on extraction yield, and the presence of water in hydroalcoholic solvents, while beneficial for polar compounds, may reduce the solubility of semi-polar or non-polar bioactive components, diluting their bioactivity and concentration.

Secondly, the drying regime, temperature, and time, may have an impact on the stability and extraction yield of the bioactive compounds dramatically. Increased temperatures during drying may lead to degradation of temperature-sensitive compounds, and uneven drying conditions may introduce variability in extraction yield and phytochemical content. The possible loss of volatile compounds during drying may also increase the supposed phytochemical value of the extracts. The study also fails to account for the optimization of extraction time and temperature, which are determinants of maximum yield and compound integrity preservation. In Soxhlet extraction, extended heating may degrade temperature-sensitive compounds, compromising the quality of the final extract. All these limitations emphasize the need for further optimization and a wider approach in future research.

## 6. Conclusions

This study underscores the significant role of both extraction method and solvent choice in determining the biological activity of plant extracts. Soxhlet extraction using alcohol (MTAS) was found to be the most effective

method for obtaining bioactive compounds with strong anthelmintic properties, likely due to the increased extraction efficiency and the solubility of potent bioactive compounds in alcohol. Hydroalcoholic extracts, while effective, showed slightly lower bioactivity, possibly due to reduced solvent efficiency. The findings suggest that Soxhlet extraction with alcohol is optimal for maximizing the anthelmintic potential of *Marsdenia tenacissima*.

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