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Synergistic Effects of Phenanthroindolizidine Alkaloids from *Tylophora indica* Against *Ascaridia galli*: Phytochemical Characterization and *In vitro* Evaluation

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ABSTRACT

The increasing resistance of helminths such as *Ascaridia galli* to conventional anthelmintics has necessitated the search for alternative treatments from natural sources. This study aimed to assess the anthelmintic properties of phenanthroindolizidine alkaloids derived from *Tylophora indica* leaves, a plant renowned for its medicinal value, including its traditional use in treating respiratory disorders, inflammation, and various infections. The leaves were subjected to sequential extraction with chloroform, ethanol, and water, which produced yields of 7.2%, 17.8%, and 11.2%, respectively. Phytochemical analysis revealed that the ethanol extract was rich in bioactive compounds, including significant amounts of alkaloids, flavonoids, phenols, and terpenoids. Quantitative analysis confirmed the ethanol extract's superiority, displaying the highest contents of phenolics (7.51 ± 0.62 mg/g), flavonoids (9.34 ± 1.63 mg/g), and alkaloids (17.65 ± 1.69 mg/g), underscoring its potential for various therapeutic applications. Further fractionation and High-Performance Liquid Chromatography (HPLC) purification isolated key phenanthroindolizidine alkaloids, including Tylophorinidine, Tylophorine, Septicine, Tylophorinol, and Antofine. Structural characterization via Nuclear Magnetic Resonance (NMR) and High-Resolution Mass Spectrometry (HRMS) validated these compounds. *In vitro* assays demonstrated significant dose-dependent anthelmintic activity against *Ascaridia galli* worms. Ethanol extracts exhibited the highest mortality rates, achieving 100% mortality within 24 hours at a concentration of 5 mg/mL. The mixture of all five alkaloids at 500 µg/mL showed a synergistic effect, leading to rapid and complete anthelmintic action. The egg embryonation assay further highlighted the efficacy of these alkaloids. The egg embryonation assay further demonstrated the potent efficacy of these alkaloids, with the mixture at 500 µg/mL inhibiting 92.67% of egg development, surpassing the positive control, i.e. piperazine citrate, which showed 87.25% inhibition. Among individual alkaloids, Tylophorinidine exhibited the highest inhibition of egg embryonation (80.76%), followed by Antofine (78.42%). These findings demonstrated the potent anthelmintic properties of phenanthroindolizidine alkaloids from *Tylophora indica*, particularly when used in combination (Tylophorinidine, Tylophorine, Septicine, Tylophorinol, and Antofine), compared to their individual effects. The study underscores the potential of these compounds as effective treatments for helminth infections and highlights the importance of further research to isolate specific mechanisms and optimize their therapeutic efficacy.

Keywords: Anthelmintic activity, *Ascaridia galli*, Phenanthroindolizidine alkaloids, Phytochemical analysis, *Tylophora indica*

INTRODUCTION

Parasitic infections pose significant challenges to the poultry industry worldwide, with *Ascaridia galli* (*A. galli*) being a prevalent nematode that detrimentally affects poultry health and productivity (Permin et al., 1999). Ascaridiasis, the disease caused by *A. galli*, results in poor feed conversion, reduced growth rates, and significant economic losses (Mitiku et al., 2023). Conventional treatments for nematode infections in poultry have predominantly relied on synthetic anthelmintics (Coles et al., 2006). However, the overuse and misuse of these drugs have led to the development of resistant parasite strains, raising concerns about their long-term efficacy and safety (Kaplan, 2004). This situation underscores the urgent need for alternative, sustainable anthelmintic solutions. While several natural products have been investigated for their anthelmintic properties, the potential of plant-derived alkaloids as effective and safe alternatives to synthetic anthelmintics has gained significant attention in recent years (Katiki et al., 2019; Molgaard et al., 2021). Alkaloids, a diverse class of nitrogen-containing compounds found in various plants, have demonstrated broad-spectrum anthelmintic activity against a wide range of parasites (Waller et al., 2001). Their mechanisms of action often involve disrupting neuromuscular function, inhibiting enzymes crucial for parasite survival, or inducing oxidative stress (Katiki et al., 2019). Furthermore, their complex chemical structures contribute to reduced risks of drug resistance development, while their relatively low toxicity to hosts enhances their appeal as viable anthelmintic agents (Wink, 2012; Molgaard et al., 2021).

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Moreover, the utilization of plant-derived alkaloids aligns with the growing interest in sustainable and environmentally friendly pest control methods (Katiki et al., 2019). Despite the investigation of numerous natural products for anthelmintic activity, the potential of plant-derived alkaloids, particularly phenanthroindolizidine alkaloids (PAs) from *Tylophora indica*, remains largely untapped for combating *Ascaridia galli* infections. The existing gap in the literature regarding the synergistic effects of these alkaloids necessitates further investigation. *Tylophora indica*, a medicinal herb native to India, is well-known for its wide range of therapeutic properties, including anti-inflammatory, immunomodulatory, and anticancer activities (Sarma et al., 2011). Phenanthroindolizidine alkaloids derived from this plant, such as tylophorine and tylophorinine, have shown remarkable bioactivity in numerous studies (Wu et al., 2001; Choudhary et al., 2004). These alkaloids, however, have not been extensively studied for their anthelmintic potential against nematodes like *A. galli*. The primary objective of this study was to evaluate the anthelmintic efficacy of a combination of phenanthroindolizidine alkaloids isolated from *Tylophora indica* leaves against *Ascaridia galli*. This involved detailed phytochemical analysis and structural characterization of the alkaloids, followed by *in vitro* evaluation of their synergistic anthelmintic effects. It is hypothesized that the combination of phenanthroindolizidine alkaloids from *Tylophora indica* exhibits a synergistic anthelmintic effect against *Ascaridia galli*, enhancing efficacy compared to individual alkaloid treatments.

MATERIALS AND METHODS

Plant material

The leaves of *Tylophora indica* (Asclepiadaceae) were collected near Osmania University, Hyderabad, India, in March 2021. After inspection and thorough rinsing, they were air-dried in the shade to preserve phytochemicals and then ground into fine powder using a mixer grinder, preparing them for subsequent analysis and extraction.

Extraction

200 grams of powdered leaf material were sequentially extracted with chloroform, ethanol, and water using a Soxhlet apparatus to ensure thorough compound extraction. For each solvent (350 mL), the mixture was heated to 50°C and refluxed for 8 hours. This temperature was chosen to prevent the degradation of heat-sensitive compounds while ensuring efficient extraction. The resulting extracts were filtered to remove solid residues and then evaporated under reduced pressure at 40°C to obtain crude extracts. These extracts were subsequently stored at 4°C to preserve their integrity. This stepwise extraction process effectively utilized chloroform, ethanol, and water as solvents, maximizing the recovery of diverse compounds from the plant material (Handa et al., 2008).

Qualitative analysis of phytochemicals in *Tylophora indica* leaves

Tylophora indica leaf powder underwent qualitative analysis for the identification of alkaloids, flavonoids, phenols, saponins, steroids, terpenoids, glycosides, tannins, carbohydrates, and amino acids, following the protocol described by Harborne (1998).

Quantitative phytochemical analysis

Total phenolic content

The total phenolic content (TPC) of *Tylophora indica* leaf extracts was evaluated using the Folin-Ciocalteu assay (Prior et al., 2005). A solution containing 1 mL of the *Tylophora indica* extract (1 mg/mL) was combined with 2.5 mL of 10% Folin-Ciocalteu reagent and 2 mL of 2% sodium carbonate solution. After incubation for 15 minutes, the absorbance was recorded at 765 nm using a microplate reader. A calibration curve for gallic acid (ranging from 1 to 0 mg/mL) was utilized, and the results were reported as gallic acid equivalents (GAE) per milligram of dry extract weight. All measurements were conducted in triplicate to ensure consistent and reliable data.

Total flavonoid content

The total flavonoid content (TFC) of *Tylophora indica* leaf extracts was assessed using a colorimetric aluminum chloride method (Chun et al., 2003). A 0.5 mL aliquot of the extract was mixed with methanol, 10% aluminum chloride, potassium acetate, and water. The mixture was incubated for 30 minutes in a dark environment. The absorbance of the solution was then measured at 420 nm using a UV-1650 spectrophotometer (Shimadzu, Japan). A standard curve for quercetin (ranging from 1 to 0 mg/mL) was prepared, and the findings were expressed in quercetin equivalents (QE) per gram of dry extract weight. Each sample was analyzed in triplicate to ensure accuracy and reproducibility (Prior et al., 2005).

Total alkaloidal content

The quantification of alkaloids in *Tylophora indica* leaf extracts was achieved through a precise colorimetric assay. To prepare the samples, one gram of the extract was solubilized in a 2% sulfuric acid solution prepared with 50% ethanol, followed by filtration and sequential extraction with chloroform. The chloroform phase was evaporated to dryness, and the residue was reconstituted in 2% sulfuric acid. This solution was then reacted with bromocresol green in the presence of a phosphate buffer adjusted to pH 4.7. Further chloroform extraction was performed, and the absorbance of the solution was recorded at 470 nm using a UV-visible spectrophotometer. Atropine served as the calibration standard, and the results were represented as atropine equivalents (AE) in milligrams per gram of dry sample weight. All experiments were conducted in triplicate to ensure reproducibility and minimize error (Prior et al., 2005).

Total triterpenoid content

The determination of triterpenoid concentration was carried out according to the methodology proposed by Lei et al. (2015). In this procedure, 1 mL of the crude extract obtained from *Tylophora indica* leaf powder was combined with 1 mL of 5% (w/v) vanillin dissolved in glacial acetic acid and 2 mL of perchloric acid. The mixture was maintained at 60°C for 15 minutes and subsequently cooled in an ice bath for 20 minutes. Following this, 10 mL of acetic acid was introduced, and the solution was homogenized. After an additional incubation period of 10 minutes, the absorbance of the reaction mixture was measured at 538 nm using a spectrophotometer. Calibration was performed with oleanolic acid as the reference standard, and the results were expressed in terms of oleanolic acid equivalents (OAE) per milligram of dry extract weight.

Collection and maintenance of *Ascaridia galli*

Adult *Ascaridia galli* worms were obtained from the intestines of naturally infected chickens, sourced from local poultry farms and slaughterhouses in Hyderabad, India. Chickens were identified as infected based on clinical signs and post-slaughter visual inspection of intestines for *Ascaridia galli*. Upon collection, the worms were promptly transferred to Tyrode's solution or physiological saline, maintained at 37°C. To minimize temperature fluctuations, insulated containers were used during the transfer process, ensuring a stable environment for the worms. Continuous monitoring indicated that temperature variations were minimal, staying within a range of ± 2°C during collection and transfer. These measures were essential to preserve the physiological integrity and viability of the worms. The worms were stored in these solutions for a brief period to guarantee their suitability for subsequent experimental tests. Careful and precise handling of the worms was paramount for accurate in vitro experimentation (Brewer and Wehr, 2001).

Observation of motility, morbidity, and mortality of *Ascardia galli*

Ten live and active *Ascaridia galli* worms were placed in separate airtight sterile containers. Each container was filled with 25 mL of Ringer Locke (RL) solution mixed with *Tylophora indica* solvent extracts (chloroform, ethanol, and aqueous) at concentrations of 0.5, 1, 2, 3.5, and 5 mg/mL. A control group was maintained in the Hedon-Fleig solution without any extract addition. Piperazine citrate, serving as the positive control, was utilized at a concentration of 5 mg/mL. Behavioral responses of the worms were meticulously monitored at predetermined temporal intervals (5, 15, 30 minutes, and 1, 2, 4, 8, 15, and 24 hours) and systematically categorized based on motility: highly vigorous (++++), moderate (+++), reduced (++), minimal (+), or complete cessation of movement (-). The outcomes of this detailed observational framework provided the foundational data for subsequent in vitro experimental evaluations (Tavares et al., 2013).

Quantitative measure of mortality percentage

The reduction in motility of *Ascaridia galli* worms exposed to chloroform, ethanol, and aqueous leaf extracts of *Tylophora indica* was quantitatively assessed using an Electronic Micro-Motility Meter (EMM) or Optical Multimeter, as described by Gadelhaq et al. (2016). This instrument operates by detecting interruptions in a light beam caused by the locomotor activity of worms within a quartz chamber containing Ringer Locke (RL) solution. These interruptions are translated into electrical signals by a photodetector, enabling real-time quantification of motility inhibition, expressed as a percentage reduction. Experimental conditions were rigorously standardized with a temperature controller maintaining environmental stability. The three extracts (chloroform, ethanol, aqueous) were evaluated in groups of ten worms per extract, with each group tested in triplicate at concentrations of 1, 2, 3, 4, and 5 mg/mL in Petri dishes at ambient temperatures ranging between 25-30°C. Observations of worm motility were conducted at defined intervals of 1, 5, 10, 15, and 24 hours. Additionally, the experimental design incorporated a positive control (5 mg/mL piperazine citrate in RL) and a negative control (RL solution alone) to validate the outcomes. The quantitative analysis highlighted the extracts' potential anthelmintic activity by showing significant motility suppression in treated worms compared to controls, providing valuable insights into the efficacy of *Tylophora indica* leaf extracts against *A. galli*.

Gas chromatography-mass spectrometry analysis of *Tylophora indica* leaves

The ethanolic extract of *Tylophora indica* (100 mg) was dissolved in 1 mL of HPLC-grade methanol and spiked with 1 µL of 2,4,6-trimethylphenol (1 mg/mL) as an internal standard. After vortexing and filtration through a 0.45 µm PTFE syringe filter, 100 µL of the filtrate was derivatized with 50 µL of MSTFA (N-methyl-N-[trimethylsilyl] trifluoroacetamide) at 60°C for 30 minutes. The analysis was conducted using an Agilent 7890 GC system coupled to an Agilent 7000D Triple Quadrupole GC-MS/MS, fitted with an HP-5MS (High Performance-5 Methyl Silicone) capillary column (30 m x 0.25 mm, 0.25 µm film thickness; Agilent Technologies). Helium (99.999%) was used as the carrier gas at 1 mL/min. The oven temperature was programmed to start at 70°C (2 minutes), ramp to 150°C at 10°C/min (1 minute), and then to 280°C at 5°C/min (10 minutes). Mass spectrometry was performed in electron impact mode with anion source, quadrupole, and transfer line temperatures set at 230°C, 150°C, and 280°C, respectively. The instrument operated in MRM mode across an m/z range of 50–550. Peak identification relied on retention times and NIST library (v2017) spectra, while quantification used internal standard calibration. System performance was validated with blank methanol and standard solutions, and repeatability was confirmed via triplicate injections, ensuring precise and reproducible results. The mass range was 50–550 m/z, and data were collected in multiple reaction monitoring (MRM) mode. Data analysis involved calibration using standards and identifying molecular ion peaks based on retention times and mass spectra. Quantification was performed using the internal standard method, where the peak area ratios of analytes to the internal standard (2,4,6-trimethylphenol) were used to calculate the concentrations of the target compounds. The quality control included analyzing blank methanol and standard solutions to confirm system performance. Repeatability tests validated the reliability of results, ensuring robust analysis (Patel et al., 2011).

Solvent fractionation

The ethanolic extract of *Tylophora indica* leaf powder was fractionated systematically. Approximately 10 grams of crude extract were diluted in 100 mL of distilled water and placed in a separatory funnel. The aqueous solution was first extracted with 100 mL of hexane to isolate non-polar substances, with the hexane fraction collected separately. The aqueous layer was then extracted three times with 100 mL of ethyl acetate to capture ethyl acetate-soluble compounds. The ethyl acetate fractions were combined and concentrated via vacuum distillation at 40°C. Next, the residual aqueous layer was extracted three times with 100 mL of n-butanol. The n-butanol fractions were pooled and concentrated under reduced pressure at 40°C, yielding the n-butanol fraction. The final aqueous layer, devoid of hexane-, ethyl acetate-, or n-butanol-soluble compounds, was concentrated at 40°C under reduced pressure to produce the aqueous fraction till one-tenth of the original volume. All fractions-hexane, ethyl acetate, n-butanol, and aqueous were dried and stored individually at 4°C for subsequent analysis. This method effectively separated the ethanolic extract into distinct fractions of varying polarities, enabling detailed chemical and biological evaluations (Koleva et al., 2001).

Isolation of phenanthroindolizidine alkaloids from ethyl acetate fraction

The phenanthroindolizidine alkaloids were isolated from the ethyl acetate fraction of the *Tylophora indica* leaf extract through a systematic process. The ethyl acetate fraction was concentrated under a vacuum to a crude residue and purified via silica gel column chromatography (60 cm × 3 cm, 2 mL/min flow rate) using a chloroform/ethanol/diethylamine gradient (10:0:0.05 to 5:2:0.05 v/v/v). Fractions were analyzed by TLC with UV and alkaloid-specific stains, pooled, and further purified by preparative HPLC on a Waters XBridge C18 column (250 mm × 10 mm, 5 µm particle size). HPLC purification utilized a methanol/water gradient (50:50 to 80:20 v/v over 30 minutes) to separate alkaloids based on polarity and stationary phase affinity. Methanol optimized alkaloid solubility, while water maintained initial polarity. A 5 mL/min flow rate and 500 µL–1 mL injection volumes ensured efficient resolution and high-purity isolation for further analysis. UV detection at 254 nm was used for alkaloid identification. The entire process was conducted at room temperature. The purified phenanthroindolizidine alkaloids were identified and confirmed through ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopy, with spectral data compared to established standards. This method effectively extracted and purified phenanthroindolizidine alkaloids from the ethyl acetate fraction of *Tylophora indica* leaf extract (Dhiman et al., 2013).

Effect of phenanthroindolizidine alkaloids on motility, morbidity, and mortality

All experimental procedures involving *Ascaridia galli* worms adhered to institutional and national ethical guidelines for animal research. Ethical approval for this study was obtained from PGP Life Sciences, with an approval number of PGP/LS/77/042/2023. Ten live and active *Ascaridia galli* worms were placed in sterile containers, which were tightly sealed and filled with 25 mL of Hedon-Fleig solution. The worms were treated with either Tylophorinidine (TLD), Tylophorine (TLP), Septicine (SPN), Tylophorinol (TNL), or Antofine (ANF) at concentrations of 250, 500, and 1000

µg/mL, or with a combination of all alkaloids in the ratio of 1:1:1:1:1 (500 µg/mL). A control group was maintained in a Hedon-Fleig solution without any alkaloids, and a positive control piperazine citrate was used at 2.5 mg/mL. The behavior of the worms was periodically observed at 5, 15, and 30 minutes, and at 1, 2, 4, 8, 15, and 24 hours. Activity levels were classified as very active (++++), moderately active (+++), somewhat active (++), sluggish (+), or deceased (-). The subsequent *in vitro* analyses were based on the findings of this observational study (Tavares et al., 2013).

Effect of phenanthroindolizidine alkaloids on mortality

An Electronic Motility Meter (EMM) was employed to quantitatively evaluate the mortality of *Ascaridia galli* worms treated with phenanthroindolizidine alkaloids TLD, TLP, SPN, TNL, and ANF at concentrations of 250, 500, and 1000 µg/mL, as well as a combined treatment of all five alkaloids in a 1:1:1:1:1 ratio (500 µg/mL), following the protocol outlined in Section 2.7 on quantitative percentage mortality measurement. Motility inhibition measurements were taken at 1, 5, 10, 15, and 24 hours post-treatment. A control group was maintained in a Hedon-Fleig solution without alkaloids, while piperazine citrate was used at 2.5 mg/mL. The EMM provided direct measurements of the inhibition percentage of motility for each group, facilitating a quantitative evaluation of the anthelmintic effects of the alkaloid mixture on both strains of *Ascaridia galli* (Gadelhaq et al., 2016).

Evaluation of phenanthroindolizidine alkaloids on *Ascaridia galli* egg embryonation

A modified *in vitro* egg embryonation assay was used to assess the effects of phenanthroindolizidine alkaloids on piperazine-sensitive and piperazine-resistant *Ascaridia galli* strains. Egg viability was confirmed microscopically by identifying intact eggs with clear shells and motile contents. Egg suspensions (100 viable eggs in 0.15 mL PBS) were prepared using flotation with a saturated salt solution, followed by PBS washing. The suspensions were mixed with phenanthroindolizidine alkaloids (TLP, TLD, SPN, TNL, or ANF) at 250, 500, and 1000 µg/mL or a combination of all five (500 µg/mL, 100 µg/mL each). Piperazine citrate (2.5 mg/mL) and PBS served as positive and negative controls, respectively. Mixtures were distributed into 96-well plates and incubated at 30°C ± 1°C for 12 days in a temperature-controlled incubator with daily monitoring. Each treatment was performed in triplicate for statistical reliability. Post-incubation, egg developmental stages were assessed microscopically using established morphological criteria to classify eggs as embryonated or unembryonated. The percentage of unembryonated eggs was recorded for each group up to day 12. This method, based on a modified assay from (Author, Year), quantified embryonation inhibition by observing the absence of cellular division or embryonic structures under 40× magnification, ensuring precise evaluation of treatment effects (Gauly et al., 2001).

Statistical analysis

Data were presented as mean ± SD. The statistical significance of differences between groups was analyzed using one-way ANOVA followed by the Turkeys post hoc test. GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) was used for the analysis, and a significant difference was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Extraction and yield

Sequential Soxhlet extraction of *Tylophora indica* leaves resulted in three distinct crude extracts including a dark green, viscous chloroform extract; a brownish, gummy ethanol extract; and a dark brown, solid water extract. The yields of extracts from *Tylophora indica* leaves were calculated after rotary evaporation, resulting in 7.2% for chloroform, 17.8% for ethanol, and 11.2% for aqueous extraction.

Phytochemical analysis

The qualitative phytochemical analysis of *Tylophora indica* leaves identified various bioactive compounds in chloroform, ethanol, and aqueous extracts, with results summarized in Table 1. The ethanol extract was the richest in phytochemicals, suggesting it was a superior solvent for extraction. Alkaloids were the most abundant in the ethanol extract of *Tylophora indica*, while flavonoids and glycosides were the highest in the aqueous extract. Saponins were present in chloroform and ethanol but not in water, indicating solubility differences. Steroids preferred the non-polar chloroform solvent. Terpenoids and phenolic compounds, recognized for their extensive biological activities and potent antioxidant properties, were identified in the ethanol and aqueous extracts of *Tylophora indica*. Tannins were detected exclusively in the chloroform extract, while carbohydrates and amino acids, critical for their nutritional significance, were present across all extracts, with the highest concentrations observed in the aqueous fraction. Lipid content was predominantly associated with the chloroform extract. These findings highlight the intricate phytochemical composition

of *Tylophora indica* and emphasize the pivotal influence of solvent selection on the extraction efficiency of bioactive compounds. Among the solvents tested, ethanol demonstrated superior efficacy in extracting a broad spectrum of phytoconstituents, reflecting its versatility as an extraction medium.

Table 1. Phytochemical analysis of *Tylophora indica* leaves

Number	Phytochemical compounds	Chloroform	Ethanol	Aqueous
1	Alkaloids	-	+++	+
2	Flavonoids	-	++	+++
3	Glycosides	-	++	++
4	Saponins	+	+	-
5	Steroids	++	+	-
6	Terpenoids	-	+	+
7	Phenols	-	++	++
8	Tannins	+	-	-
9	Carbohydrates	+	+	+++
10	Amino acids	+	+	++
11	Fats	++	-	-

+++: Abundant; ++: Considerable; +: Present; -: Absent

Quantitative phytochemical analysis

The quantitative phytochemical analysis of *Tylophora indica* leaves revealed significant variations in the content of phenolics, flavonoids, alkaloids, and triterpenoids across different solvent extracts (Table 2). The total phenolic content (TPC) was quantified using the Folin-Ciocalteu reagent assay, revealing that the aqueous extract exhibited the highest TPC (8.13 ± 0.77 mg/g), followed by the ethanol extract (7.51 ± 0.62 mg/g) and the chloroform extract (3.21 ± 0.28 mg/g, $p < 0.05$). These findings highlight the superior efficacy of highly polar solvents in extracting phenolic compounds, consistent with previous studies (Harborne et al., 1999; Prior et al., 2005). Similarly, the total flavonoid content (TFC) was determined using an aluminum chloride-based colorimetric method, which indicated the aqueous extract had the highest TFC (13.24 ± 1.06 mg/g), followed by the ethanol extract (9.34 ± 1.63 mg/g) and chloroform extract (1.01 ± 0.11 mg/g, $p < 0.05$). These results suggest that the high phenolic and flavonoid concentrations in the aqueous extract may correlate with significant antioxidant potential (Harborne, 1998; Chun et al., 2003). The ethanol extract exhibited the highest total alkaloidal content (17.65 ± 1.69 mg/g, $p < 0.05$), highlighting its potential for pharmacological potential for therapeutic applications such as antimicrobial, anti-inflammatory, and anthelmintic effects (Thawabteh et al., 2019). The total triterpenoid content, quantified using the vanillin-glacial acetic acid method, was greatest in the aqueous extract (4.94 ± 0.25 mg/g), followed by ethanol (4.21 ± 0.26 mg/g) and chloroform (0.89 ± 0.05 mg/g), underscoring its therapeutic potential. These findings demonstrated that ethanol and aqueous extracts are particularly effective for extracting bioactive compounds from *Tylophora indica* leaves, with notable antioxidant and pharmacological properties.

Table 2. Quantitative phytochemical analysis of *Tylophora indica* leaves

Phytochemical class	Chf Ex.	EtOH Ex.	Aq. Ex
Total phenolic content (mg/g Gallic acid equivalent)	3.21 ± 0.28	7.51 ± 0.62	8.13 ± 0.77*
Total flavonoid content (mg/g Rutin equivalent)	1.01 ± 0.11	9.34 ± 1.63	13.24 ± 1.06*
Total alkaloidal content (mg/g atropine equivalents)	2.54 ± 0.18	17.65 ± 1.69$^{\#}$	5.64 ± 0.32
Total triterpenoids content (mg/g Oleanolic acid equivalent)	0.89 ± 0.05	4.21 ± 0.26*	4.94 ± 0.25*

The data are presented as mean ± standard deviation (SD) from three independent experiments. Different superscripts indicate significant differences between the extracts for each phytochemical class. Statistical significance was set at $p < 0.05$. Specifically, the superscript * denotes a significant difference compared to Chf. Ex., while $^{\#}$ denotes a significant difference compared to both Chloroform and Aqueous extracts.

Observation of motility, morbidity, and mortality of *Ascardia galli*

The observation of motility, morbidity, and mortality of *Ascaridia galli* worms treated with various extracts of *Tylophora indica* demonstrated significant differences in their observed effects based on the type and concentration of the extract as shown in Table 3. The control group maintained very high activity levels (++++) throughout the observation period. In contrast, the ethanol extract showed the highest efficacy in reducing worm motility, with complete inactivity (-) observed at concentrations of 3.5 mg/mL and above within 24 hours. Ethanol is superior for anthelmintic activity due to its intermediate polarity, enabling the extraction of both polar and non-polar bioactive compounds like

alkaloids, phenolics, and flavonoids. It effectively disrupts plant cell walls for efficient extraction and is safe and compatible with biological assays. The aqueous extract also demonstrated significant anthelmintic activity, with the worms becoming completely inactive at a concentration of 5 mg/mL within 24 hours., compared to chloroform extract. The chloroform extract was less effective compared to the ethanol and aqueous extracts, with partial inactivity observed at the highest concentration of 5 mg/mL. The positive control group treated with piperazine citrate showed a rapid reduction in worm activity, becoming completely inactive (-) within 8 hours, affirming the reliability of the assay. The data suggested that the ethanol extract of *Tylophora indica* is the most potent in terms of anthelmintic activity, followed by the aqueous extract. The results of this study are consistent with prior research demonstrating the efficiency of ethanol as a solvent for extracting bioactive constituents with notable pharmacological activities (Harborne, 1998). The observed outcomes highlight the potential of *Tylophora indica* extracts as potent anthelmintic agents, with the ethanol extract exhibiting the most pronounced efficacy. Further investigations are essential to isolate and structurally characterize the active constituents contributing to this activity and to elucidate their underlying mechanisms of action.

Table 3. Visual inspection of motility, morbidity, and mortality of *Ascardia galli* over the exposure to different solvent extracts of *Tylophora indica* leaves

Solvent extracts	Conc. in mg/ml	5 minutes	15 minutes	30 minutes	1 hour	2 hours	4 hours	8 hours	15 hours	24 hours
Control	0	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)
Chl. ex	0.5	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)
	1	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)
	2	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)
	3.5	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
	5	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)	(++)
EtOH. ex	0.5	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)	(+++)	(++)
	1	(+++)	(+++)	(++)	(++)	(++)	(+)	(+)	(+)	(-)
	2	(++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)
	3.5	(++)	(++)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
	5	(++)	(+)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Aq. ex	0.5	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)
	1	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)	(+++)	(+++)
	2	(+++)	(+++)	(+++)	(+++)	(++)	(++)	(++)	(++)	(++)
	3.5	(+++)	(++)	(++)	(++)	(++)	(++)	(+)	(+)	(-)
	5	(++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)
Piperazine	2.5	(+++)	(++)	(++)	(-)	(-)	(-)	(-)	(-)	(-)

Very active (++++); Moderately active (+++); Slightly active (++); Sluggish (+); Dead (-); Chl. Ex: Chloroform extract, MeOH: Methanolic extract; Aq: Aqueous extract of *Tylophora indica*.

Quantitative measure of mortality percentage

The data presented in Figure 1 indicate that the ethanol extract demonstrated superior efficacy in inducing mortality in *Ascaridia galli* worms compared to all other treatment groups. At a concentration of 5 mg/mL, the ethanol extract achieved complete mortality (100%) within 5 hours, with this effect remaining consistent over a 24-hours observation period ($p < 0.05$). Low concentrations of ethanol extract also showed substantial mortality, with 60% and 70% mortality observed at concentrations of 3 mg/mL and 4 mg/mL within an hour, eventually reaching 100% by 24 hours. The aqueous extract showed moderate efficacy, with the highest concentration (5 mg/mL) causing 50% mortality at 24 hours ($p < 0.05$). Lower concentrations resulted in lesser mortality, indicating a dose-dependent effect. The chloroform extract was the least effective, with only 20% mortality observed at the highest concentration (5 mg/mL) after 24 hours. Lower concentrations exhibited minimal or no mortality. The results indicated that the ethanol extract of *Tylophora indica* is the most potent in causing mortality of *Ascaridia galli* worms, followed by the aqueous extract, and the chloroform extract showing the least efficacy. The high mortality rate observed with the ethanol extract suggests it contains the most effective bioactive compounds for anthelmintic activity, consistent with previous studies demonstrating the superior extraction capabilities of ethanol for obtaining pharmacologically active compounds (Harborne, 1998).

GC-MS analysis of *Tylophora indica* leaves

The ethanolic extract of *Tylophora indica* leaves underwent GC-MS/MS analysis, with the resulting chromatogram and the list of identified compounds displayed in Figure 2 and Table 4, respectively. The analysis identified a total of 37 chemical constituents in the extract, including prominent compounds such as β-Sitosterol, Stigmasterol, Lupeol, and notable flavonoids like Quercetin and Kaempferol, as detailed in Table 1. These compounds indicated potential therapeutic applications of *Tylophora indica*, particularly in anti-inflammatory, antioxidant, and anti-cancer activities (Patel et al., 2011). Notably, significant quantities of phenanthroindolizidine alkaloids, including Tylophorine,

Tylophorinidine, Tylophorinol, Antofine, Cryptopleurine, and Septicine, were detected. Tylophorinidine, the most abundant alkaloid, constituted 80.36% of the area, highlighting its major presence in the ethanolic extract. This compound, along with Tylophorine and Tylophorinol, has been extensively studied for its potent anti-cancer properties (Saraswati et al., 2013). Antofine and Septicine also showed substantial presence, further contributing to the therapeutic potential of the extract. The identification of flavonoids, such as Quercetin and its derivatives, supports the antioxidant capabilities of the extract. These findings underscored the rich phytochemical composition of *Tylophora indica* leaves and highlighted the efficacy of ethanol as a solvent for extracting bioactive compounds, particularly phenanthroindolizidine alkaloids. This comprehensive profiling through GC-MS/MS provides a valuable foundation for further pharmacological studies and potential drug development. The presence of these compounds suggests that the ethanolic extract of *Tylophora indica* leaves could be a valuable source of therapeutic agents for treating inflammatory diseases and cancers. Further studies are warranted to isolate these alkaloids and investigate their specific mechanisms of action.

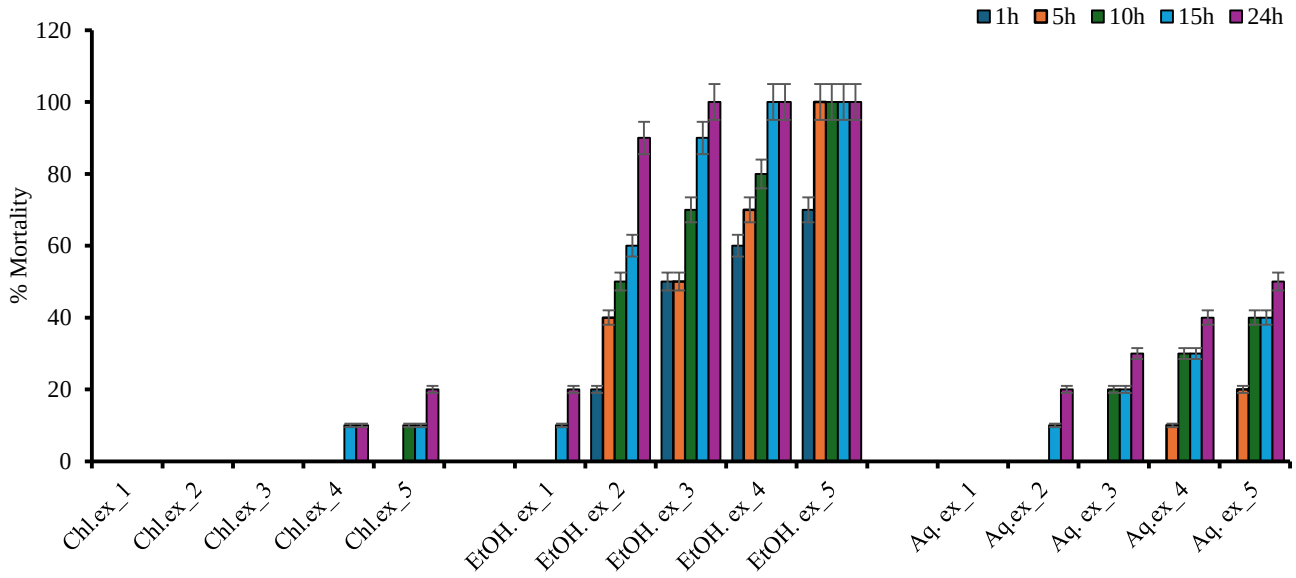

Figure 1. Mortality of *Ascaridia galli* after exposure to different concentrations (1-5 mg/ml) of *Tylophora indica* leaf extracts (chloroform, ethanol, and aqueous) at different time points (1h, 5h, 10h, 15h, and 24h). Data presented as mean percentage mortality. A statistically significant difference was observed between all groups, with a p-value of less than 0.05

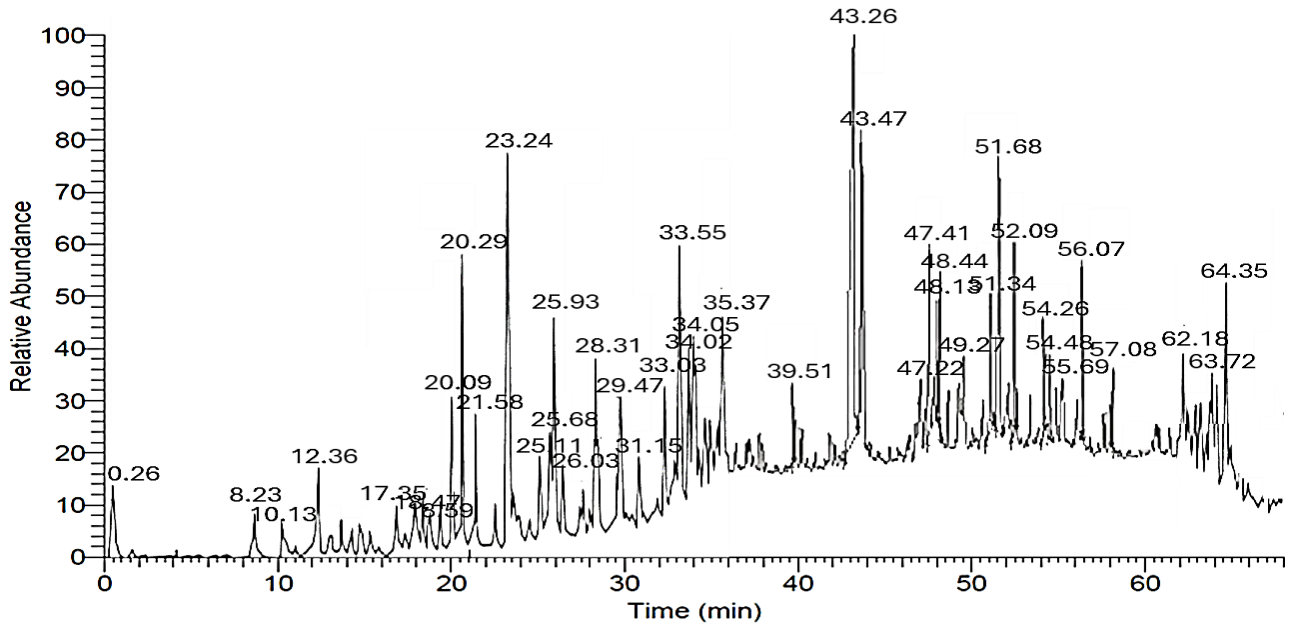

Figure 2. The GC-MS chromatogram of the ethanolic extract of *Tylophora indica* leaves illustrates the detection of diverse phytochemical constituents. The x-axis denotes the retention time (minutes), while the y-axis represents the relative abundance as a percentage. Each peak in the chromatogram corresponds to a distinct compound eluting at a specific retention time, with the relative abundance of the peaks reflecting the proportional representation of the respective compounds within the extract.

Table 4. GC-MS analysis of ethanolic extract of *Tylophora indica* leaves

Number	R_t	Name of a chemical compound	Molecular formula	M. Wt (g/mol)	Area (%)
1	0.26	Cyclotrisiloxane, Hexamethyl-	$C_6H_{18}O_3Si_3$	222.46	13.46
2	12.36	Tert-Butyl(5-Isopropyl-2-Methylphenoxy) Dimethylsilane	$C_{16}H_{26}O_2Si$	278.47	18.42
3	20.09	Benzene, 2-[(Tert-Butyldimethylsilyl) Oxy]-1-Isopropyl-4-Methyl-	$C_{16}H_{26}OSi$	262.46	30.24
4	20.29	1,4-Bis (Trimethylsilyl)Benzene	$C_{12}H_{22}Si_2$	238.48	58.54
5	21.58	2,4-Cyclohexadien-1-One, 3,5-Bis(1,1-Dimethylethyl)-4-Hydroxy-	$C_{17}H_{26}O_2$	262.39	26.68
6	23.24	Methyl β-Sitosterol	$C_{30}H_{53}O$	429.71	77.34
7	25.11	Methyl (Methyl 4-O-Methyl-Alpha-D-Mannopyranoside) Uronate	$C_{10}H_{18}O_8$	266.25	17.51
8	25.68	Phytol	$C_{20}H_{40}O$	296.53	24.19
9	25.93	n-Hexadecanoic Acid	$C_{16}H_{32}O_2$	256.42	45.63
10	26.03	Tocopherol	$C_{29}H_{50}O_2$	430.71	16.39
11	28.31	Lupeol	$C_{30}H_{50}O$	426.71	35.84
12	29.47	Campesterol	$C_{28}H_{48}O$	400.68	26.73
13	31.15	Stigmasterol	$C_{29}H_{48}O$	412.69	14.96
14	33.03	β-Sitosterol	$C_{29}H_{50}O$	414.71	33.14
15	33.55	α-Amyrin	$C_{30}H_{50}O$	426.71	58.42
16	34.02	β-Sitosterol glycoside	$C_{35}H_{60}O_6$	592.85	40.42
17	34.05	Epicatechin	$C_{15}H_{14}O_6$	290.27	41.27
18	35.37	Protocatechuic acid	$C_7H_6O_4$	154.12	42.61
19	39.51	Gallic acid	$C_7H_6O_5$	170.12	31.73
20	43.26	Tylophorine	$C_{24}H_{25}NO_2$	359.46	100
21	43.47	Tylophorinidine	$C_{23}H_{23}NO_2$	345.43	80.36
22	47.22	Ferulic acid	$C_{10}H_{10}O_4$	194.19	29.83
23	47.41	Tylophorinol	$C_{24}H_{26}N_2O_2$	374.48	55.08
24	48.13	Vanillic acid	$C_8H_8O_4$	168.15	48.97
25	48.44	Antofine	$C_{24}H_{27}NO_4$	393.48	51.66
26	49.27	p-Coumaric acid	$C_9H_8O_3$	164.16	33.15
27	51.34	Cryptopleurine	$C_{19}H_{21}NO$	263.37	44.62
28	51.68	Septicine	$C_{21}H_{23}NO$	305.41	74.69
29	52.09	Quercetin	$C_{15}H_{10}O_7$	302.24	54.38
30	54.26	Kaempferol	$C_{15}H_{10}O_6$	286.24	42.11
31	54.48	Rutin	$C_{27}H_{30}O_{16}$	610.52	39.42
32	55.69	Isorhamnetin 3-O-β-glucoside	$C_{22}H_{22}O_{12}$	462.4	33.71
33	56.07	Isorhamnetin 3-O-β-galactoside	$C_{22}H_{22}O_{11}$	446.4	50.44
34	57.08	Quercetin 3-O-β-glucuronide-4′-methylether	$C_{22}H_{20}O_{13}$	508.4	31.09
35	62.18	Kaempferol-3-O-glucoside	$C_{21}H_{20}O_{12}$	448.38	35.11
36	63.72	Kaempferol-3,7-dirhamnoside	$C_{27}H_{30}O_{14}$	594.53	28.74
37	64.35	Quercetin 7-O-β-D-glucopyranoside	$C_{21}H_{20}O_{12}$	464.38	45.36

R_t: Retention time; M.Wt: Molecular Weight

Solvent fractionation, HPLC purification, and structural analysis of phenanthroindolizidine alkaloids

The solvent fractionation of the ethanolic extract of *Tylophora indica* leaves successfully separated the extract into hexane, ethyl acetate, n-butanol, and aqueous fractions. Each fraction was collected, concentrated, and stored for subsequent analysis. The hexane fraction targeted non-polar substances, while the ethyl acetate and n-butanol fractions captured compounds with intermediate polarities. The remaining aqueous fraction contained the polar compounds. This fractionation method allowed for a detailed examination of the chemical and anthelmintic properties of each fraction, facilitating a comprehensive analysis of the phytochemical constituents of *Tylophora indica* (Koleva et al., 2001). Given the promising anthelmintic activity observed in the ethyl acetate fraction, phenanthroindolizidine alkaloids were successfully isolated from this fraction of the *Tylophora indica* leaf extract through a combination of column chromatography and preparative HPLC. The systematic isolation process began with concentrating the ethyl acetate fraction under reduced pressure to obtain a residue. This residue was subjected to column chromatography using a chloroform/ethanol/diethylamine gradient, and the fractions collected were monitored with TLC. Fractions indicating the presence of phenanthroindolizidine alkaloids were pooled and further purified using preparative HPLC. The HPLC chromatograms (Figures 3a and 3b) show distinct peaks corresponding to the isolated phenanthroindolizidine alkaloids, including Tylophorine, Tylophorinidine, Septicine, Tylophorinol, and Antofine. Figure 3a (Standard alkaloid Run Chromatogram) shows clear, distinct peaks for known phenanthroindolizidine alkaloids, including Tylophorinidine, Tylophorine, Septicine, Tylophorinol, and Antofine, with retention times (R_t) at 4.35 min, 7.38 min, 9.55 min, 12.29 min and 15.36 min, respectively. These retention times serve as reference points for comparing the sample run. Figure 3b (Sample Run Chromatogram) confirms the presence of the above phenanthroindolizidine alkaloids by displaying corresponding peaks at nearly identical retention times (Rt) to the expected elution order. Additionally, the sample run chromatogram includes an extra peak at 23.16 min, indicating the presence of an unknown compound. The consistency of retention times across both runs verifies the accurate identification of Tylophorinidine, Tylophorine, Septicine, Tylophorinol, and Antofine in the sample. The phenanthroindolizidine alkaloids were further identified and verified using ^{1}H-NMR, ^{13}C-NMR, and HRMS spectroscopy. The spectral data matched established standards, confirming the accurate identification of the isolated compounds. The major alkaloids identified included Septicine, Tylophorinol, and Antofine, which are known for their potent pharmacological activities, particularly anti-inflammatory and anti-cancer properties. Spectral data was illustrated here.

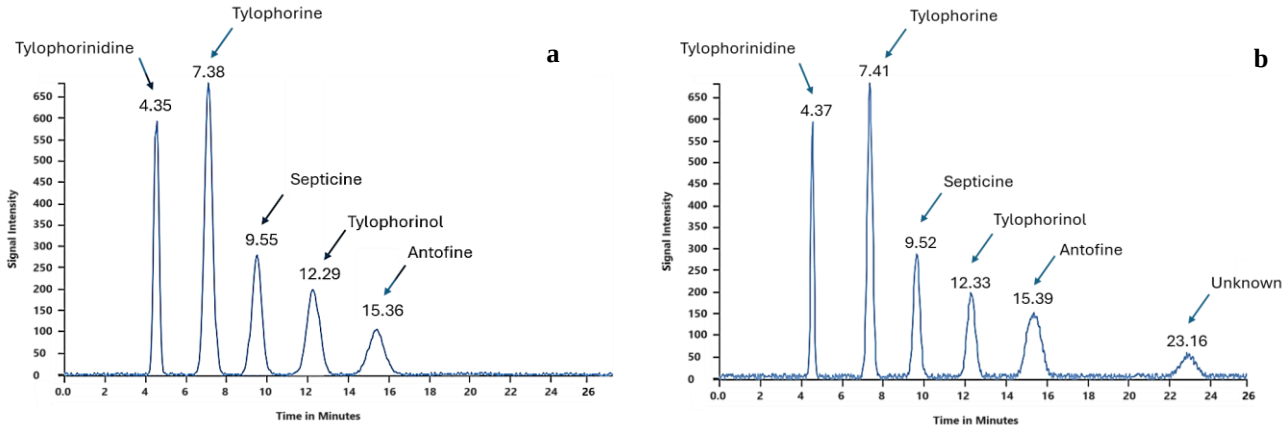

Figure 3. HPLC Chromatograms of Standard Phenanthroindolizidine Alkaloids and *Tylophora indica* Leaf Extract Fraction. **a**: HPLC chromatogram of standard phenanthroindolizidine alkaloids (Tylophorine, Tylophorinidine, Septicine, Tylophorinol, and Antofine) separated under defined chromatographic conditions. **b**: HPLC chromatogram of the ethyl acetate fraction of *Tylophora indica* leaf extract, further purified to isolate phenanthroindolizidine alkaloids.

Effect of phenanthroindolizidine alkaloids on motility, morbidity, and mortality

The study evaluated the impact of phenanthroindolizidine alkaloids from *Tylophora indica* on *Ascaridia galli* motility as shown in Table 5. The control group maintained high activity levels throughout the 24 hours. TLD and TLP exhibited dose-dependent motility inhibition, with TLD showing complete inactivity at 1000 µg/mL by 24 hours and TLP at 500 µg/mL and 1000 µg/mL showing rapid reduction in motility. SPN and TNL also demonstrated potent anthelmintic effects, achieving complete inactivity at 1000 µg/mL by 24 hours. ANF showed similar results with a significant reduction in motility at higher concentrations. The mixture of alkaloids at 500 µg/mL exhibited the strongest synergistic effect, with complete inactivity by 1 hour. The positive control group with piperazine citrate confirmed the assay's reliability, showing complete inactivity by 15 hours. These findings suggested that phenanthroindolizidine alkaloids, particularly Tylophorinidine and Tylophorine, possess significant anthelmintic activity and potential as effective therapeutic agents.

Table 5. Visual inspection of motility, morbidity, and mortality of *Ascardia galli* over the exposure of different phenanthroindolizidine alkaloids of *Tylophora indica*

Solvent extracts	Conc. in mg/ml	5 minutes	15 minutes	30 minutes	1 hour	2 hours	4 hours	8 hours	15 hours	24 hours
Control	0	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)	(++++)
TLP	250	(++++)	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)	(++)
	500	(+++)	(+++)	(+++)	(++)	(++)	(+)	(+)	(-)	(-)
	1000	(++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)
TLD	250	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)	(++)	(++)
	500	(+++)	(+++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)
	1000	(++)	(++)	(+)	(+)	(-)	(-)	(-)	(-)	(-)
SPN	250	(++++)	(++++)	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)
	500	(+++)	(+++)	(++)	(++)	(++)	(++)	(+)	(+)	(-)
	1000	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
TNL	250	(++++)	(++++)	(++++)	(++++)	(++++)	(+++)	(+++)	(+++)	(+++)
	500	(+++)	(+++)	(++)	(++)	(++)	(++)	(+)	(++)	(+)
	1000	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
ANF	250	(++++)	(++++)	(++++)	(++++)	(++++)	(+++)	(+++)	(++)	(++)
	500	(+++)	(+++)	(++)	(++)	(++)	(+)	(+)	(-)	(-)
	1000	(+++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)	(-)
Mixture of PA's	(1:1:1:1:1) 500	(+++)	(++)	(+)	(-)	(-)	(-)	(-)	(-)	(-)
Piperazine	2.5	(+++)	(+++)	(++)	(++)	(+)	(+)	(+)	(-)	(-)

Conc: Concentration; Very active (++++); Moderately active (+++); Slightly active (++); Sluggish (+); Dead (-).

Effect of phenanthroindolizidine alkaloids on mortality

The mortality data reveal that phenanthroindolizidine alkaloids exhibit significant anthelmintic activity against *Ascaridia galli*, with effectiveness being dose-dependent as shown in Figure 4. Higher concentrations resulted in greater mortality rates specifically, TLD at 250 µg/mL induced 20% mortality by 24 hours, achieving 100% mortality at 500 µg/mL and 1000 µg/mL by 15 hours and 10 hours, respectively, indicating high efficacy at higher concentrations. TLP showed a similar trend, with 10% mortality at 250 µg/mL by 24 hours and reaching 100% mortality at 1000 µg/mL by 10 hours. SPN and TNL demonstrated substantial efficacy, with SPN at 1000 µg/mL reaching 100% mortality by 15 hours and TNL achieving the same by 15 hours. Lower concentrations of these compounds were less effective but still caused notable mortality rates. Antofine (ANF) was similarly effective, with 100% mortality at 1000 µg/mL by 15 hours. The mixture of all five alkaloids at 500 µg/mL exhibited the most rapid and complete anthelmintic effect, achieving 100% mortality by 10 hours, suggesting a possible synergistic effect. The positive control, piperazine citrate (2.5 mg/mL), achieved 100% mortality by 15 hours, confirming the reliability of the assay. These findings indicated that phenanthroindolizidine alkaloids from *Tylophora indica* possess potent anthelmintic properties, with Tylophorinidine, Tylophorine, and the alkaloid mixture showing the most promise. The dose-dependent mortality observed underscores their potential as effective treatments for helminth infections. Further studies are recommended to isolate and characterize the specific mechanisms underlying their anthelmintic activity.

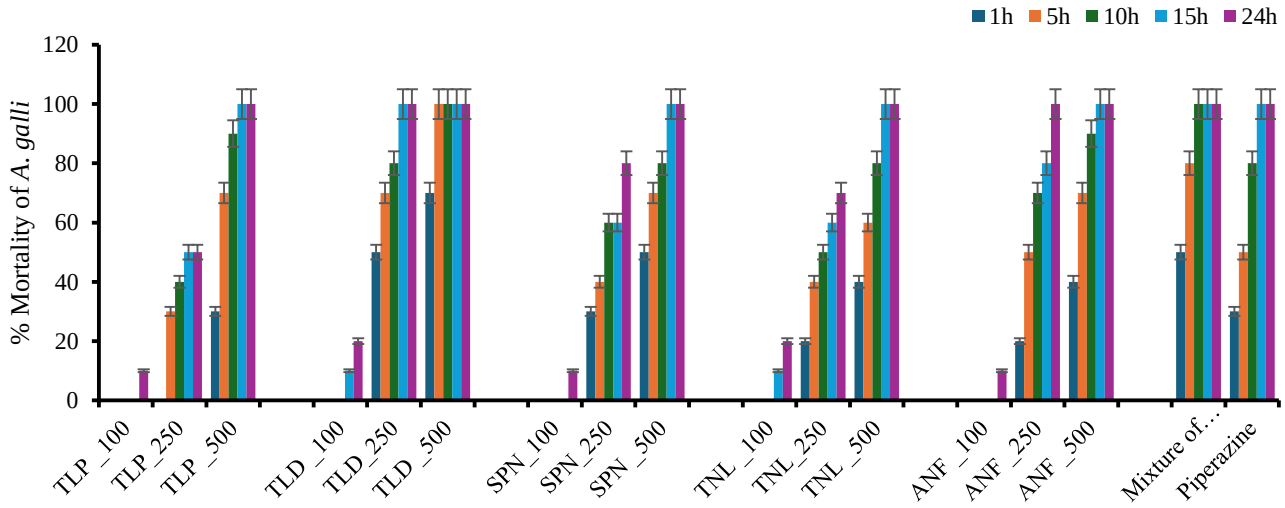

Figure 4. Percentage mortality of *Ascaridia galli* following exposure to varying concentrations (100-500 µg/mL) of TLP, TLD, SPN, TNL, and ANF, a mixture of all five alkaloids, or piperazine (positive control) at different time points (1, 5, 10, 15, and 24 hours). A statistically significant difference was observed between all groups, with a p-value of less than 0.05.

Evaluation of phenanthroindolizidine alkaloids on *Ascaridia galli* egg embryonation

The *in vitro* egg embryonation assay results demonstrated that phenanthroindolizidine alkaloids from *Tylophora indica* exhibited significant inhibitory effects on the embryonation of *Ascaridia galli* eggs (Figure 5, $p < 0.05$). TLD at 1000 µg/mL showed the highest inhibition among the individual alkaloids, with 80.76% undeveloped eggs, followed by ANF at 78.42%, TNL at 76.89%, SPN at 74.11%, and TLP at 72.81% ($p < 0.05$). The mixture of all five alkaloids at 500 µg/mL demonstrated the highest embryonation inhibition, with 92.67% undeveloped eggs, suggesting a synergistic effect that enhances their overall efficacy. The positive control, piperazine citrate (2.5 mg/mL), showed 87.25% inhibition, confirming the reliability of the assay and providing a benchmark for comparison. The results underscore the potential of phenanthroindolizidine alkaloids as promising anthelmintic agents, demonstrating dose-dependent inhibition of egg embryonation, which emphasizes their therapeutic applicability in controlling helminth infections. Further investigation is necessary to isolate and elucidate the specific mechanisms responsible for their inhibitory effects on embryonation, as well as to evaluate potential synergistic interactions among these alkaloids to enhance their efficacy.

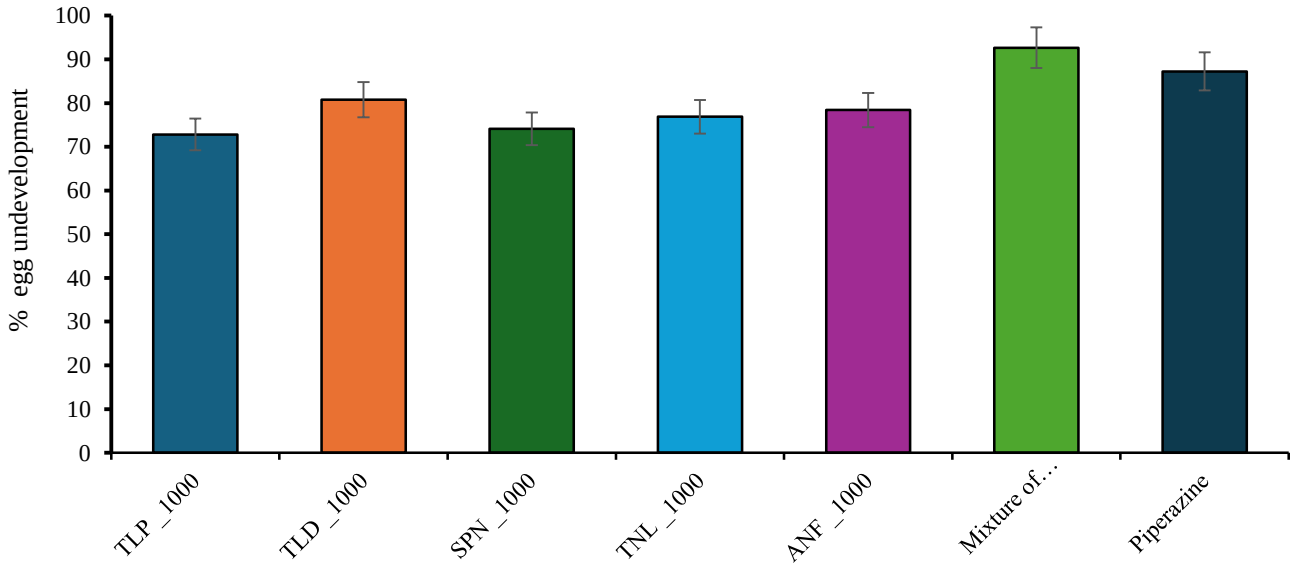

Figure 5. Percentage inhibition of egg development in *Ascaridia galli* following exposure to 1000 µg/mL of TLP, TLD, SPN, TNL, ANF, a mixture of all five alkaloids, or piperazine (positive control). A statistically significant difference was observed between all groups, with a p-value of less than 0.05.

CONCLUSION

Structural characterization identified five phenanthroindolizidine alkaloids: Tylophorinidine, Tylophorine, Septicine, Tylophorinol, and Antofine, all of which exhibited significant dose-dependent anthelmintic activity against *Ascaridia galli*. The combination of all five alkaloids demonstrated a synergistic effect, achieving the highest motility inhibition, mortality rates, and embryonation inhibition (92.67% at 500 µg/mL). Among the individual compounds, Tylophorinidine and Antofine were particularly potent. These findings establish the potential of *Tylophora indica* alkaloids as promising therapeutic agents for helminth infections. Future studies should focus on isolating specific active compounds, elucidating their mechanisms of action, and further exploring synergistic interactions to optimize their efficacy.

DECLARATIONS

Availability of data and materials

All data generated or analyzed during this study are included in the manuscript. Additional datasets, if required, are available from the corresponding author upon reasonable request.

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Competing interests

The authors have not declared any conflict of interest.

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Authors' contributions

Yadagiri Katti was responsible for conceptualization, methodology, investigation, data analysis, drafting, and revising the manuscript. Bethi Neeraja contributed to conceptualization, supervision, resource provision, and critical manuscript review and editing. Both authors have read and approved the final version of the manuscript before publication in the present journal.

Ethical considerations

All authors have contributed to the preparation of this original paper. The authors observed the final version of the finished paper and evaluated any corrections and updates. They also checked the similarity index of the article.

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