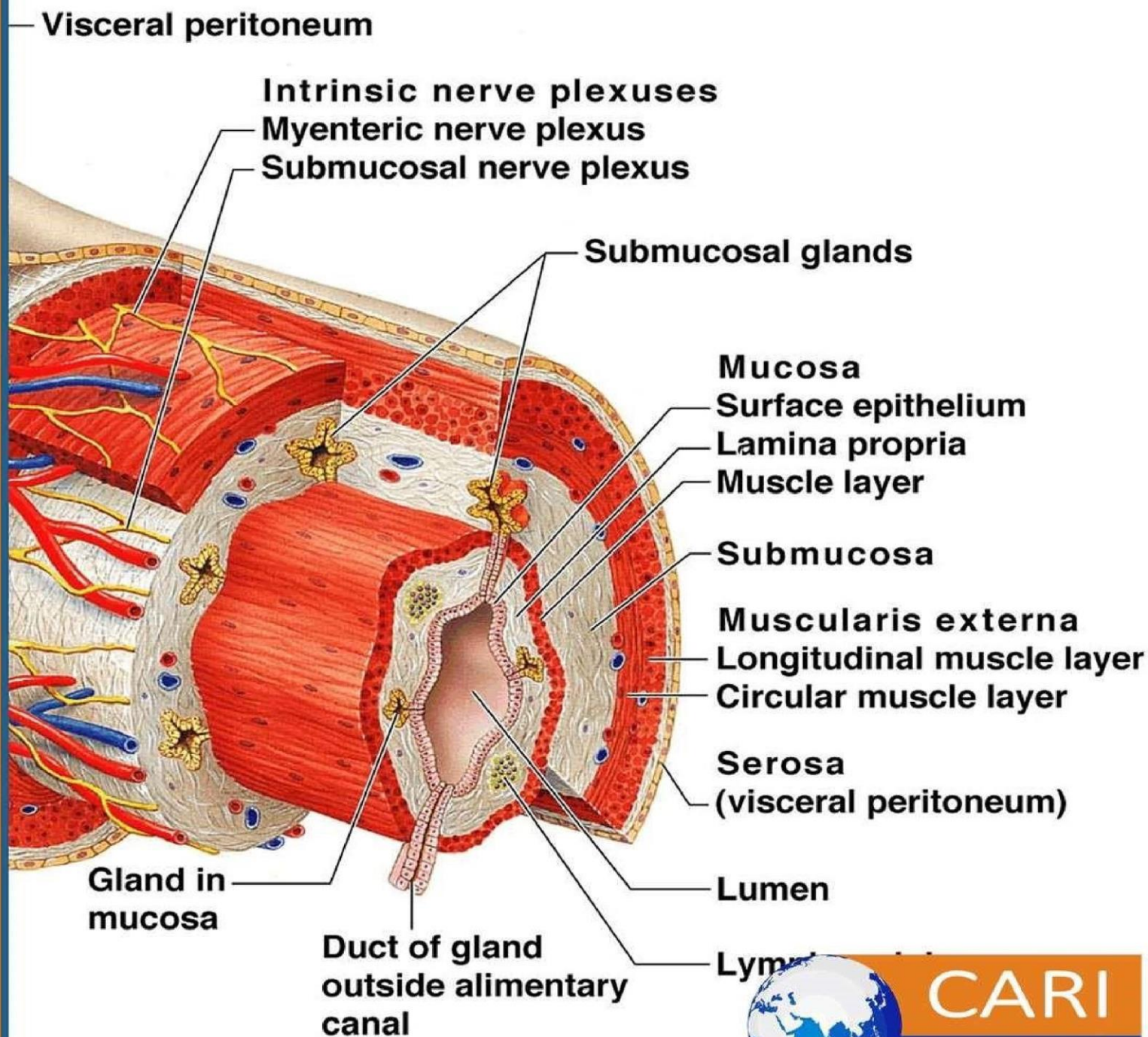



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**Bioactive Phytochemicals and Solvent Extraction Efficiency of
Justicia betonica: Anthelmintic Applications against *Schistosoma
mansoni* Cercariae in Kisii County, Kenya**



Bioactive Phytochemicals and Solvent Extraction Efficiency of *Justicia betonica*: Anthelmintic Applications against *Schistosoma mansoni* Cercariae in Kisii County, Kenya

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Abstract

Purpose: The anthelmintic activity of *Justicia betonica* (*J. betonica*) against *Schistosoma mansoni* is of significant interest, owing to ethnobotanical investigations that highlight the plants diverse phytochemical composition. However, comprehensive metabolomic profiling is required to link its chemical profile to its potential in reducing the global schistosomiasis burden. This study aimed to evaluate the in vitro anthelmintic potential of *J. betonica* against *Schistosoma mansoni* (*S. mansoni*) cercariae.

Methodology: An in vitro comparative experimental study was conducted at the KEMRI NTDs Laboratory in Kisumu, Kenya. *J. betonica* extracts were prepared using different solvents and screened against *S. mansoni* cercariae at concentrations ranging from 0.25 to 1.0 mg/mL using a 24-well plate assay. Praziquantel and distilled water served as positive and negative controls, respectively. Cercarial motility and mortality were monitored over 1 hour. Dose-response analysis was performed to determine LC₅₀, EC₅₀, and Minimum Inhibitory Concentration (MIC) values.

Findings: The extracts exhibited a dose-dependent effect on cercarial mortality. At the highest concentration (1.0 mg/mL), the ethanol and methanol extract achieved the highest mean mortality (mean=10) and the strongest time-dependent activity, achieving complete mortality (100%) within 40 minutes. The calculated LC₅₀ was 0.12mg/ml, while the MIC was determined to be 0.25mg/ml. Statistical analysis indicated a significant difference in mortality rates compared to the negative control ($p < 0.001$), with the efficacy of the highest dose being comparable to Praziquantel. *J. betonica* extracts demonstrate significant anti-schistosomal activity in vitro, effectively inducing cercarial mortality at low concentrations.

Unique Contribution to Theory, Policy and Practice: These findings suggest that *J. betonica* is a promising candidate for further phytochemical isolation and development as a plant-based anthelmintic agent. Also it provides scientific validation for its traditional medicinal use, contribute to knowledge on plant-derived anti-parasitic agents, support policy initiatives promoting natural-product research, and offer a foundation for future drug development efforts.

Keywords: *Justicia Betonica*, *Schistosoma Mansoni*, *Phytochemicals*, *Cercaricidal Activity*

1.0: Introduction

Helminth infections remain a significant global public health concern, particularly in tropical and subtropical regions where environmental and social economic conditions favor transmission (1). Among these infections, schistosomiasis caused by *Schistosoma mansoni* continues to affect millions of people worldwide and contributes significantly to malnutrition, anemia, impaired cognitive development, and reduced productivity (1). In Kenya, schistosomiasis is highly prevalent in areas surrounding major water bodies and regions with limited sanitation infrastructure (2). Kisii County has reported persistent cases of intestinal schistosomiasis, especially among communities relying on natural water sources for domestic and agricultural activities (3). Despite the availability of synthetic anthelmintic drugs such as praziquantel, prolonged and widespread use has raised concerns regarding reduced efficacy, possible drug resistance, toxicity, and environmental residues (4,5&6).

Consequently, there is increasing interest in identifying alternative, affordable, and environmentally sustainable anthelmintic agents from medicinal plants. Medicinal plants are rich sources of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids, many of which possess antiparasitic and anthelmintic activities. These phytochemicals act through different mechanisms, including disruption of parasite metabolism, inhibition of energy production, and damage to the tegumental structure of helminths (7&8). Previous studies have demonstrated that plant-derived extracts exhibit significant activity against various helminths in both in vitro and in vivo models, supporting their potential as alternatives to conventional drugs (9&4).

Justicia betonica is one of the medicinal plants widely utilized in traditional medicine in different parts of Africa, including Kenya, for the management of gastrointestinal disorders, parasitic infections, inflammation, and microbial diseases. In Kisii County, local communities traditionally use the plant for treating intestinal ailments and related infections, indicating its ethnopharmacological relevance. However, despite its widespread traditional application, limited scientific information exists regarding its phytochemical composition, extraction efficiency using different solvents, and specific anthelmintic activity against *S. mansoni* cercariae. The biological activity of plant extracts is strongly influenced by the extraction solvent and processing conditions, since solvents differ in their ability to isolate bioactive compounds based on polarity and chemical properties. Evaluating solvent extraction efficiency is therefore essential for optimizing the recovery of therapeutically important phytochemicals and improving the pharmacological potential of medicinal plants. Advances in phytochemical profiling and bioassay-guided fractionation have further enhanced the identification and validation of bioactive compounds responsible for therapeutic effects.

Therefore, this study aimed to evaluate the phytochemical composition, solvent extraction efficiency, and anthelmintic activity of *J. betonica* extracts against the cercarial stage of *S.*

mansoni in Kisii County, Kenya. The study hypothesized that *J. betonica* contains bioactive phytochemicals with significant cercaricidal activity and that extraction solvents influence the yield and efficacy of these compounds. Findings from this study may contribute to the development of effective, affordable, and environmentally sustainable plant-based anthelmintic agents for the control of schistosomiasis and related helminth infections.

2.0: Materials and Methods

2.1: Study area and design

This study was conducted at the Neglected Tropical Diseases (NTDs) Laboratory of the Kenya Medical Research Institute (KEMRI) in Kisumu, Kenya. An in vitro comparative experimental design was employed to evaluate the antischistosomal activity of *Justicia betonica* crude extracts prepared using different solvents, as well as their potential synergistic effects against *Schistosoma mansoni* cercariae. Praziquantel (0.25 mg/mL) and distilled water served as positive and negative controls.

2.2: Collection and preparation of plant material

Fresh samples of *J. betonica* were collected from Keumbu, Kisii County, Kenya, and authenticated by taxonomists at the National Museums of Kenya. The plant materials were dried under the shade for one month, pulverized into fine powder, and stored for extraction.

2.3: Extraction of plants materials

Extraction was carried out using acetone, ethanol, methanol, and distilled water following standard solvent extraction procedures (10). Briefly, 50 g of powdered material was soaked in 1 L of solvent for 24 hours at room temperature. The mixtures were filtered using Whatman No. 1 filter paper and re-extracted until colourless filtrates were obtained. Filtrates were concentrated using a rotary evaporator and further air-dried to remove residual solvents. The dried extracts were weighed to determine percentage yield and reconstituted to a concentration of 1 mg/mL for bioassays.

2.4: Phytochemical screening

Qualitative phytochemical analysis of *J. betonica* extracts was performed using standard procedures (11). The presence of alkaloids, flavonoids, tannins, saponins, phenolics, glycosides, steroids, terpenoids, and quinones was determined based on characteristic colour changes or precipitate formation and recorded as present (+) or absent (-).

2.5: Collection and preparation of *S. mansoni* cercaria

Snails were collected from the shores of Lake Victoria at Dunga Beach, Kisumu County, and screened for cercarial shedding at KEMRI. *Biomphalaria sudanica* snails infected with *S. mansoni* were identified morphologically, and cercarial shedding was induced using indirect sunlight exposure. Cercariae were collected, assessed for viability based on motility, and used within 2–4 hours post-shedding.

2.6: Experimental bioassay

Bioassays were conducted using 24-well plates containing 10 cercariae per well. The cercariae were exposed in triplicate to varying concentrations of plant extracts (0.25, 0.50, 0.75, and 1.0 mg/mL). Control groups consisted of praziquantel as the positive control and distilled water as the negative control. The plates were incubated at 37°C for 1 hour to facilitate interaction between the cercariae and the plant extracts. Observations were made at 10-minute intervals for 1 hour using a dissecting microscope. Cercarial viability was assessed based on motility, morphological alterations, and mortality. Motility inhibition was determined by reduced or absent movement compared to the negative control, while morphological changes such as body deformation, tail loss, and structural damage were used as indicators of extract activity. Cercariae were considered dead when they showed complete absence of movement even after gentle stimulation. Percentage mortality and motility inhibition were calculated for each concentration and exposure time. These viability scores were subsequently used in determining LC₅₀, EC₅₀, and minimum inhibitory concentration (MIC) values. Reduced motility and structural damage were considered indicators of extract efficacy (12).

2.7: Determination of LC₅₀, EC₅₀, and MIC

LC₅₀ (lethal concentration) and EC₅₀ (effective concentration) values were determined from dose-response data, representing concentrations causing 50% mortality and 50% motility inhibition, respectively.

2.8: Data collection and analysis

Phytochemical data were recorded based on qualitative reactions, while bioassay results were based on observed mortality and motility changes. Statistical analysis was performed using SPSS version 21. Factorial ANOVA was used to assess the effects of extract concentration and exposure time, followed by Tukey's HSD, and Dunnett's post hoc tests for multiple comparisons. Statistical significance was set at $p < 0.05$.

2.9: Ethical considerations

Ethical approval was obtained from the Kisii Teaching and Referral Hospital Ethics Review Committee (Approval No. ISERC/KTRH/003/22), and a research permit was granted by NACOSTI (License No. NACOSTI/23/23265).

3.0: Results

3.1: Percentage extraction yield

Extraction yields obtained from 100 g of dried plant powder in 1 L of solvent varied substantially across the different solvent types, indicating a strong influence of solvent properties on extraction efficiency (Fig 1)

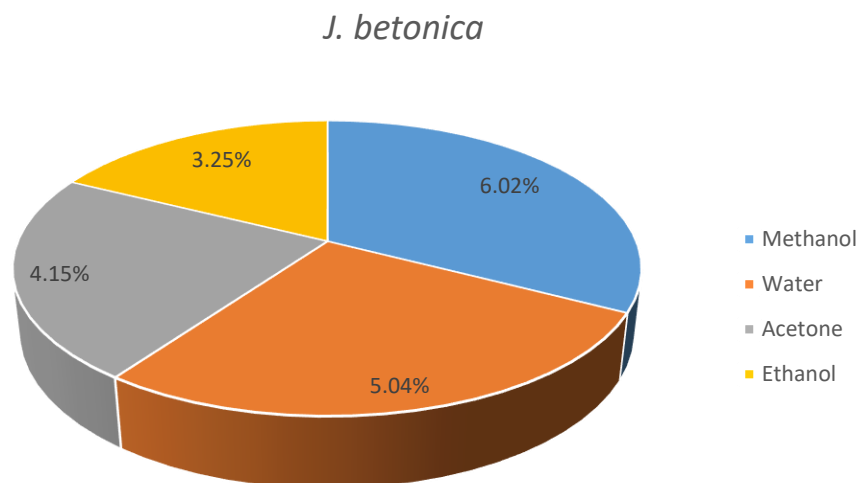


Fig 1: *Justicia betonica* crude extracts percentage yield

3.2: Phytochemical composition of *J. betonica*

A qualitative screening of *J. betonica* and the synergistic extracts, revealed the presence of various bioactive constituents across different solvent extracts. The concentrations were qualitatively assessed as + or - which were subsequently converted to numerical values ranging from 0-3 scale for qualitative analysis. Methanol and water extracts, consistently showed the highest diversity and concentration of phytochemicals across the plant species while ethanol and acetone extracts exhibited the lowest phytochemical presence (Table 1).

Table 1: Qualitative phytochemical composition of plant extracts

	Methanol solvent			Water solvent		Acetone solvent		Ethanol solvent	
	Observation	Grade		Observation	Grade	Observation	Grade	Observation	Grade
Alkaloid (wagner's test)	Red ppt	Brown +++		Brown ppt	++	Pale brown ppt	+	Pale brown ppt	+
Phenolic (Ferric Chloride test)	Blue ppt	+++		Blue green ppt	++	Pale blue	+	Pale blue	+
Flavonoids(Shinoda test)	Deep purple ppt	+++		Deep purple ppt	+++	Pale blue to purple ppt	+	Pale blue to purple ppt	+
Saponins (Froth test)	Foam 2cm ³	++		Foam 1cm ³	+	No Foam	-	Foam less than 1cm ³	+
Steroids (Salkowski test.)	Red brown ring	++		Red brown ring	++	Pale orange ring	+	Pale orange ring	+
Tannins (Ferric chloride test)	Greenish black ppt	++		Greenish black ppt	++	Greenish ppt	+	Absence of greenish ppt	-
Terpenoids: Salkowski Test	Clear interface ring	-		Clear interface ring	-	Clear interface ring	-	Clear interface ring	-
Glycosides (borntreger's test)	Clear ammonical layer	-		Clear ammonical layer	-	Clear ammonical layer	-	Clear ammonical layer	-
Quinone	No red colouration	-		No red colouration	-	No red colouration	-	No red colouration	-

Key: +++ = High, ++ = Moderate, + = Low, - = Absent

When a quantitative analysis of the plant species was done the plant demonstrated high phytochemical content, particularly in alkaloids (mean = 2.00) and flavonoids (mean = 2.00) (Fig 1)

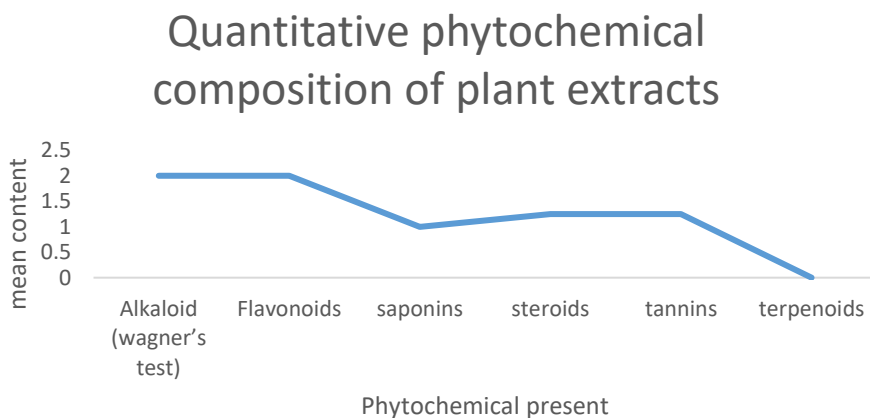


Fig 2: Quantitative phytochemical composition of plant extracts of *J.betonica*

3.3: Time-Dependent Cercaricidal Activity of *Justicia betonica* Extracts and Controls

Exposure of *Schistosoma mansoni* cercariae to various solvent extracts of *Justicia betonica* resulted in time-dependent mortality. Overall, cercaricidal efficacy correlated significantly with exposure duration across all extract types ($p < 0.001$).

In ethanol extracts, the mean interval cercariae mortality was recorded as 3.75 after 10 min of exposure, rising to 4.50 at 20 min, before declining to 1.58 at 30 min and 0.25 at 40 min; cumulative complete mortality (mean = 10.0) was achieved by the 40-min mark. Methanol extracts exhibited a comparable trend, with mean interval mortalities of 4.33, 2.58, 2.33, and 0.75 recorded at 10, 20, 30, and 40 min, respectively, ultimately reaching cumulative complete mortality (mean = 10.0) at 40 min.

Acetone extracts demonstrated moderate cercaricidal activity, yielding mean interval mortalities of 1.58 at 10 min, 2.67 at 20 min, 3.33 at 30 min, 2.00 at 40 min, and 0.50 at 50 min, with cumulative complete mortality (mean = 10.0) attained by 50 min. Similarly, aqueous extracts showed observable cercaricidal effects, with mean interval mortalities of 1.17, 3.33, 4.17, and 1.00 at 10, 20, 30, and 40 min, respectively, reaching cumulative complete mortality (mean = 10.0) at the 40-min interval.

The positive control (praziquantel) demonstrated high efficacy, producing mean interval mortalities of 1.58 at 30 min and 7.17 at 40 min, achieving cumulative complete mortality (mean = 10.0) by 40 min. Conversely, the negative control (distilled water) exhibited no cercaricidal effect, maintaining a mean mortality of 0 throughout the entire exposure period.

Overall, the ethanol and methanol extracts demonstrated the highest cercaricidal potency, particularly within the first 30 min of exposure, indicating robust, time-dependent acute toxicity. Acetone extracts exhibited moderate but less consistent interval effects, whereas aqueous extracts demonstrated a lower yet steadily progressing cumulative mortality trend over time. The complete

absence of mortality in the negative control group confirms that the observed cercaricidal activities were directly attributable to the bioactive profiles of the plant extracts rather than environmental confounding factors (Fig 2).

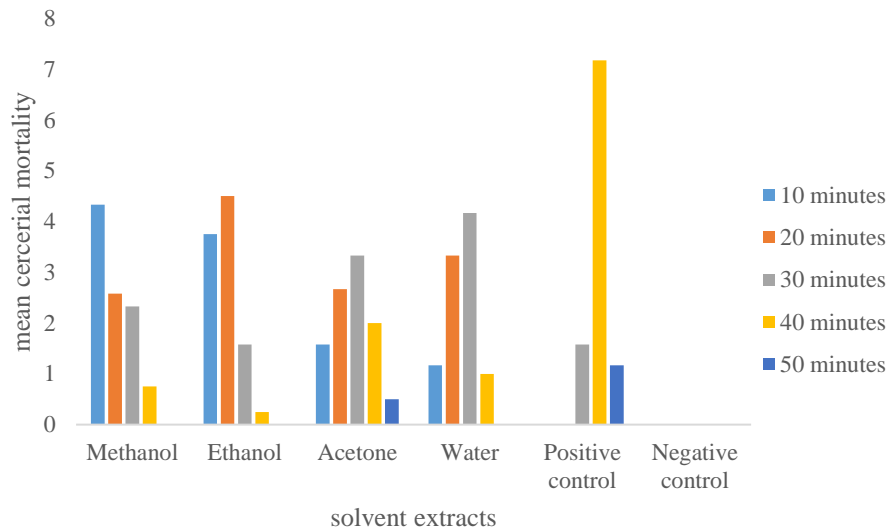


Figure 3: Time-course mortality of *S. mansoni* larvae exposed to *J. betonica* extracts, including positive control (Praziquantel) and negative control (distilled water)

3.4: Cercarial Mortality Following Exposure to *J. betonica* Extracts

Exposure of *S. mansoni* cercariae to varying concentrations of *J. betonica* extracts (0.25, 0.50, 0.75, and 1.0 mg/mL) resulted in a clear time-dependent increase in mortality, which was statistically significant ($p < 0.001$). Overall, mortality rates increased with exposure time and concentration across most solvent extracts. Mortality of *S. mansoni* cercariae increased significantly with both exposure time and extract concentration, demonstrating a combined time- and concentration-dependent cercaricidal effect of *J. betonica* extracts. Across all solvent types, mortality generally rose with increasing exposure duration. For ethanol extracts, mortality increased from 30% at 10 minutes to 60% at 20 minutes, peaking at 80% at 30 minutes and reaching 100% at 40 minutes.

Methanol extracts followed a similar trend, with mortality rising from 40% at 10 minutes to 60% at 20 minutes and 70% at 30 minutes, before achieving complete mortality (100%) at 40 minutes. Acetone extracts exhibited moderate activity, with an initial mortality of 50% at 10 minutes, although the pattern over time was less consistent. In contrast, aqueous extracts showed relatively high initial mortality (60% at 10 minutes) and a generally increasing trend throughout the exposure period. A clear concentration-dependent pattern was also observed, with higher concentrations (0.75 and 1.0 mg/mL) inducing more rapid cercarial mortality, particularly within the first 10 minutes of exposure. At 1.0 mg/mL, 70% mortality was recorded at 10 minutes, reaching 100%

by 30 minutes. At 0.75 mg/mL, complete mortality (100%) was achieved by 50 minutes. Lower concentrations exhibited slower effects; at 0.5 mg/mL, mortality was 40% at 30 minutes but reached 100% at 50 minutes, while at 0.25 mg/mL, mortality reached 90% at 40 minutes and 100% at 50 minutes.

The positive control, Praziquantel, demonstrated comparable high efficacy, with mortality increasing from 60% at 10 minutes to 70% at 20 minutes and 80% at 30 minutes, reaching 100% at 40-50 minutes. In contrast, the negative control (distilled water) showed no mortality across all time points and concentrations, confirming that the observed effects were attributable to the plant extracts rather than environmental conditions. Overall, ethanol and methanol extracts exhibited the highest cercarial mortality, particularly at earlier time points, while acetone showed moderate and variable effects. Aqueous extracts demonstrated relatively high and steadily increasing mortality. These findings confirm that *J. betonica* possesses strong cercaricidal activity that is both time and concentration-dependent (Fig 3).

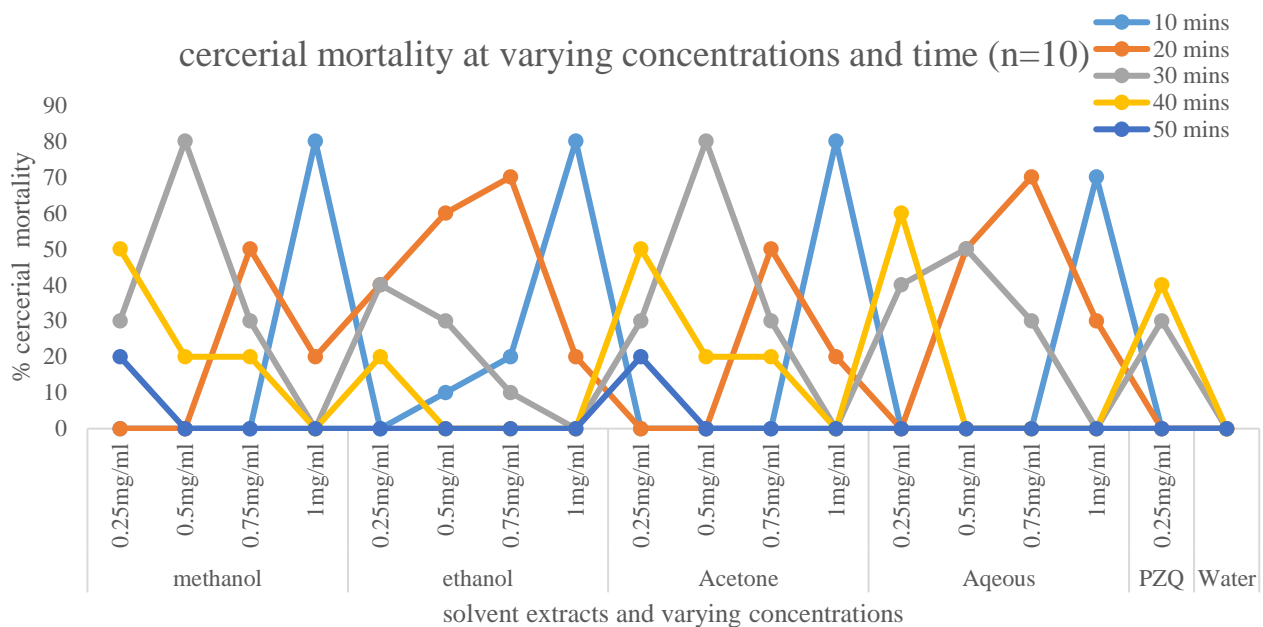


Figure 4: Cercarial mortality at varying concentrations and varying times of exposure in *J. betonica* plant extracts

3.5: Larvicidal Activity against *Schistosoma mansoni*

The methanolic extract of *J. betonica* demonstrated significant schistosomicidal activity against *S. mansoni* cercariae in a time- and concentration-dependent manner.

3.6: Cercariae Mortality and Motility Inhibition

At the highest concentration tested (1 mg/ml), immediate motility inhibition was observed, with 100% mortality achieved within 20 minutes of exposure. In contrast, lower concentrations of 0.25 mg/ml, mortality rates varied significantly across all solvent extracts and exposure times, total mortality (100%) was achieved at the end of 40 minutes in the methanol and ethanol treatment extracts, while in acetone and aqueous total mortality was achieved by the end of 50 minutes. The positive control, Praziquantel, exhibited an early mortality of 60% at 10 minutes, which declined over the subsequent interval until 100% total mortality was reached at the end of the 20 minutes. In the negative control no mortality was recorded during the entire exposure period.

3.7: Lethal Concentration (LC₅₀) of *J. betonica* Extracts

The median lethal concentration (LC₅₀), defined as the concentration required to induce 50% larval mortality at 40 minutes of exposure, was determined only for the acetone extract of *J. betonica*. This extract exhibited a LC₅₀ value of 0.12 mg/mL, indicating strong cercaricidal activity within the tested time frame. In contrast, LC₅₀ values for the remaining solvent extracts could not be reliably estimated, as their mortality responses did not consistently cross the 50% threshold across the tested concentration range under the experimental conditions. This further highlights the comparatively higher efficacy of the acetone extract in inducing cercarial mortality.

4.0: Discussion and Conclusion

The present study demonstrates that *J. betonica* exhibits strong cercaricidal activity against *Schistosoma mansoni*, with efficacy influenced by solvent type, concentration, and exposure time. The observed time- and concentration-dependent increase in mortality aligns with recent findings that plant-derived extracts exert progressive lethal effects on *S. mansoni* cercariae, particularly with increasing exposure duration and dose (13&14). Similar dose-dependent cercaricidal responses have been reported in recent phytochemical screenings, where higher extract concentrations consistently produced faster and more complete larval mortality (15&16). The ability of the acetone extract of *J. betonica* to yield a measurable LC₅₀ value (0.12 mg/mL) highlights its superior extraction efficiency for bioactive compounds responsible for cercarial mortality. This finding is consistent with recent studies showing that intermediate-polarity solvents such as acetone and methanol are more effective in isolating schistosomicidal compounds than highly polar solvents like water (16&17).

The enhanced activity is often attributed to improved solubilization of secondary metabolites such as flavonoids, alkaloids, saponins, and terpenoids, which have been widely associated with antiparasitic activity in schistosomiasis research (18&19). Comparable cercaricidal potency has been documented in other medicinal plants, where LC₅₀ values vary widely depending on species and extraction method but consistently demonstrate strong activity of bioactive fractions against *S. mansoni* cercariae. For example, recent studies report LC₅₀ values in the range of 1–300 mg/L

for various plant extracts, with acetone and methanol fractions typically showing the highest potency (13&16).

These similarities support the growing evidence that medicinal plants remain promising sources of antischistosomal agents, particularly in endemic regions where drug resistance and reinfection remain major challenges. The inability to determine LC_{50} values for the other solvent extracts suggests that their mortality responses did not consistently reach the 50% threshold within the tested concentration range. Similar limitations have been reported in recent cercaricidal screening studies, where weaker or highly polar extracts failed to produce full dose–response curves, limiting quantitative toxicological interpretation (14&15). This highlights the importance of optimizing both solvent systems and concentration ranges when evaluating plant-based schistosomicidal agents. Overall, the strong cercaricidal activity observed in *J. betonica*, particularly in the acetone extract, supports recent global evidence that plant-derived compounds are effective against early schistosome life stages and may contribute to transmission control strategies (18&19). These findings reinforce the need for further phytochemical isolation, structural characterization, and in vivo validation of active compounds to support development of novel, plant-based antischistosomal agents.

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