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ORIGINAL ARTICLE

Antimalarial Activity and Safety of *Conyza pyrrhopappa* Sch. Bip. ex A. Rich

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ABSTRACT

Background

Malaria continues to pose a significant global health challenge, particularly in developing countries including Tanzania. The rising resistance to existing antimalarial drugs has intensified the need for alternative therapeutic options.

Objective: This study aimed to evaluate the in-vivo antimalarial activity and safety profile of *Conyza pyrrhopappa*.

Methodology: Plant materials were collected from Mbulu District in Manyara Region, Tanzania. Extraction was performed using 80% ethanol-water. Fractionation was carried out via liquid-liquid partitioning with solvents of varying polarity, starting with petroleum ether, dichloromethane, and ethyl acetate. The in-vivo antimalarial activity was evaluated using a 4-day suppressive assay. Mice were infected with 2×10^7 erythrocytes infected with Plasmodium berghei ANKA. Chloroquine was used as a positive control, and extracts were administered orally. The crude extract's effects on peripheral blood mononuclear cells (PBMCs) and brine shrimps were assessed. The oral acute toxicity was tested in mice.

Results: *Conyza pyrrhopappa* extracts demonstrated significant in-vivo antimalarial activity compared with the negative control group. The highest average percentage suppression of parasitaemia (65.7%) was observed with the leaf extract at a dose of 800 mg/kg body weight. The ethyl acetate leaf fraction exhibited the highest parasitaemia suppression compared with the negative control ($P \leq .001$). In PBMCs, growth inhibition was found to be concentration-dependent, with an IC_{50} (95% CI) of 94.56 (45.82-196.8) µg/ml. In the brine shrimp lethality assay, the leaf extract exhibited an LC_{50} of 144.2 µg/ml (95% CI, 110.9 to 187.5). The oral acute toxicity test indicated that the leaf crude extract is safe, with an LD_{50}>2000 mg/kg body weight in mice.

Conclusion: *Conyza pyrrhopappa* leaf and root extracts demonstrated safety and in-vivo antimalarial activity. These findings support the traditional use of the plant in malaria treatment. Further research focusing on the ethyl acetate leaf fraction could lead to the identification of potent antimalarial compounds.

BACKGROUND

Malaria remains a major life-threatening disease in many developing countries of Sub-Saharan Africa, including Tanzania, where it continues to pose a serious public health challenge despite improvements over the past two decades. In 2023, malaria caused an estimated 597,000 deaths globally,[1] and Tanzania accounted for 4% of global malaria deaths.[2] The disease is among the leading causes of morbidity and mortality in the country, particularly affecting vulnerable groups such as children under five years of age and pregnant women. Limited access to healthcare services, favorable conditions for mosquito breeding, and socioeconomic challenges contribute to the persistence and transmission of malaria in Tanzania. Although incidence and mortality declined markedly between 2000 and 2015 due to expanded control interventions such as insecticide-treated nets and improved treatment access, Intermittent preventive treatment, progress has slowed in recent years, with persistent transmission and spatial heterogeneity across mainland regions.[2,3]

The emergence and spread of drug-resistant *Plasmodium falciparum* strains further complicate malaria control and treatment efforts. While resistance to artemisinin-based combination therapies has not yet become widespread in Africa, resistance to older antimalarial drugs such as chloroquine and sulfadoxine–pyrimethamine remains prevalent.[4] This situation significantly reduces the effectiveness of previously reliable treatments and threatens the progress made in malaria control programs.

Additionally, the adaptability of malaria parasites and the limited number of available antimalarial drug classes emphasise the vulnerability of current treatment strategies. Consequently, there is a pressing need to discover and develop new, effective, and safe antimalarial agents. Natural products, particularly those derived from medicinal plants, remain an important source of novel bioactive compounds and have historically contributed to the discovery of widely used antimalarial drugs such as quinine and artemisinin. Therefore, continued exploration of medicinal plants used in traditional medicine may provide valuable leads for the development of new antimalarial therapies.

Research into plant-based therapies has demonstrated significant potential in the search for new antimalarial agents. Numerous medicinal plants native to Tanzania have been reported to possess antimalarial properties. For instance, extracts from *Cissampelos mucronata*, *Maytenus senegalensis*, *Salacia madagascariensis*, and *Zanthoxylum chalybeum* have shown notable antiplasmodial activity in experimental screenings of Tanzanian medicinal plants.[5] However, despite their promising therapeutic potential, some plants with antimplasmodial activity have also been associated with toxicity concerns, highlighting the need for evaluation of their safety.[6,7]

Conyza pyrrhopappa is a plant species in the family Asteraceae. It is an erect herb that grows about 0.6–1.5 m tall, with branched, slightly hairy stems and alternate, narrow lanceolate leaves. The plant produces small composite flower heads with yellowish florets. It is widely distributed in tropical Africa, including countries such as Tanzania, Kenya, Uganda, Ethiopia, Rwanda, Malawi, Zambia, and the Democratic Republic of Congo. It is a medicinal plant traditionally used in local communities to manage ailments such as fever, sore throat, and diarrhea.[8] Because fever is a common symptom associated with malaria, plants used traditionally for treating febrile illnesses may contain compounds with potential antimalarial activity. Despite its traditional use, there is limited scientific evidence regarding the efficacy of *Conyza pyrrhopappa* against malaria parasites and its safety profile. Therefore, this study aimed to evaluate the antimalarial activity and safety of different extracts and fractions of *Conyza pyrrhopappa.*

METHODS

Chemicals and Reagents

Giemsa stain (Sigma®, Germany), ethanol, Methanol, Tween 80, 0.9% NaCl (normal saline), Petroleum ether, ethyl acetate and Dichloromethane were purchased from local suppliers in Tanzania. Chloroquine diphosphate was obtained from Sigma® (Steinheim, Germany).

Collection and Identification of the Plant Materials

The plant materials were collected and dried in Mbulu District, Manyara, and transported to the Institute of Traditional Medicine (ITM) as dry materials. Leaves and roots of *Conyza pyrrhopappa* were collected during two different seasons: just after the heavy rainy season and just after the light rainy season. Seasonal collection was opted to identify variations in phytochemical composition and determine the optimal harvesting time for plants with the highest levels of bioactive compounds.[9,10] The first batch, consisting of leaves only, was collected in May 2019 after the rainy season from Mbulu District, Manyara Region, and identified by a botanist, Mr Samson Hilonga, from Muhimbili University of Health and Allied Sciences (MUHAS). The collection site was recorded at GPS coordinates 36M 0758013 UMT, 9546800, 1920 m above sea level. A second batch, comprising both leaves and roots, was collected from the same location in December 2020 after the light rainy season. Plant materials obtained from the two collection periods were processed separately. Voucher specimens have been deposited in the Herbarium of the Institute of Traditional Medicine (ITM) at MUHAS under voucher number AIM-04.

Extraction of the plant materials

The plant materials were dried in the shade and ground into coarse powder, followed by extraction by the maceration method using 80% ethanol in water. The materials were placed in an amber container, and extraction solvent was added to completely cover them just above the surface and left for 48 hours with occasional agitation. They were then filtered by using cotton wool to obtain the filtrate. The filtrate was dried under reduced pressure and temperature using a rotary evaporator at 50^0 C (HAHNSHIN S&T Co. Ltd, South Korea). Complete drying was achieved using freeze-drying equipment (Genevac Ltd, Ipswich, England).

Fractionation of Crude Plant Extract

The 80% ethanolic extract was fractionated at room temperature by liquid-liquid partitioning using different solvents ranging from non-polar to polar, namely petroleum ether, dichloromethane, and ethyl acetate, as follows: About 200 g of the 80% ethanolic extract was dissolved in methanol, followed by the addition of water up to 250 ml to obtain an aqueous solution. The aqueous solution was then transferred into a separating funnel, followed by the addition of an equal volume (250 ml) of petroleum ether. The two solutions were mixed thoroughly by vigorous shaking and then allowed to stand until separation occurred, forming two distinct layers. The petroleum ether layer was separated from the aqueous layer. The process of adding petroleum ether was repeated to ensure exhaustive extraction of the nonpolar components. After fractionation with petroleum ether, the remaining aqueous solution was mixed with dichloromethane, followed by ethyl acetate, to obtain the dichloromethane and ethyl acetate fractions, respectively. At the end of the process, three fractions (petroleum ether, dichloromethane, and ethyl acetate) were obtained and concentrated using a rotary evaporator, followed by freeze drying for aqueous fractions. The extracts were stored at -20^0 C in amber glass vials until the day of analysis.

Experimental Animals

Twenty-seven young adult white albino mice, both male and female, aged 3 to 4 weeks, were obtained from the animal house of MUHAS. The animals were acclimatised to the laboratory environment, given food and water for five days before being used for the study. Throughout the study, the mice were kept in good and clean environments in aluminium cages and had unrestricted access to standard pellet laboratory food and water under

a 12-hour light/12-hour dark cycle.

Assessment of Antimalarial Activity

In-vivo antimalarial activity was assessed using Peter's four-day suppressive test as described previously.[11] Briefly, each mouse was infected by injection via the tail vein with 2 x 10^7 *P. berghei* ANKA infected red blood cells taken from donor mice with 30-35% parasitaemia, and then all mice were left for three hours. Three (3) hours post-infection, the mice were randomly allocated to different treatment groups. The groups were labelled as negative/solvent control group, positive control group and treatment groups which received single doses of the extract. The negative (solvent) control group was included to provide a baseline response in the absence of the active extract, thereby allowing any observed effects in the treatment groups to be attributed specifically to the extract rather than to the solvent or other experimental variables.

The negative control group was administered 10% Tween 80 (5 ml/kg body weight) orally, which was the solvent used to dissolve the test extracts. The positive control group received chloroquine diphosphate (10 mg/kg body weight) orally. The first treatment group was administered Dichloromethane leaf fraction (400 mg/kg body weight / day) orally, the second treatment group received Ethyl acetate leaf fraction (400 mg/kg body weight/day) orally, the third treatment group was given crude ethanolic leaf extract (400 mg/kg body weight/day) orally, and the fourth treatment group received crude ethanolic root extract (400 mg/kg body weight/day) orally. For the crude extract of plant materials collected after heavy rainfall, a dose of 800 mg/kg body weight/day was also tested. The same oral doses were administered every 24 hours for three (3) consecutive days. On the fourth day, a thin blood film was prepared from each experimental mouse for the determination of parasitaemia, stained with Giemsa and the parasites were observed at x100 under an Olympus CX31RBSF light microscope (Olympus Optical Co Ltd., Philippines).

Determination of Parasitaemia of the Mice

A drop of blood was collected from the mice by venesection of the tail and then transferred on microscope slide. The blood was spread evenly across the slide by using another slide to make a thin blood film and then allowed to dry at room temperature. The dried slides were fixed with absolute methanol for 30 seconds, dried, and then stained with 10% Giemsa stain for 30 minutes. Slides were then viewed by using Olympus CX31RBSF (Olympus Optical Co Ltd., Philippines) light microscope with oil immersion under 100x magnification. Parasitaemia was counted by using the clicker where by the Giemsa positive red blood cells were counted as malaria positive red blood cells. At least four fields of approximately 250 cells each were counted and percentage parasitaemia was determined as follows;

$$\%\ Parasitemia = \frac{Number\ of\ infected\ RBCs}{Total\ number\ of\ RBCs} x100$$

Also, the average % suppression was calculated by using the following formula

$$100\left[\frac{A-B}{A}\right]$$

Where A is the average percentage parasitaemia within the negative control group, and B is the average percentage parasitaemia within the test (treated) group. The data obtained were analysed and presented as mean ± standard deviation.

The body weight was determined by taking the average weight of mice in each test group and comparing it with that of the infected negative controls. Mortality was monitored daily, and the number of days from the time of inoculation of the parasite up to death was recorded for each mouse in the treatment and control groups throughout the follow-up period. The mean survival time (MST) for each group was calculated as given below.

$$MST = \frac{Sum\ of\ survival\ time\ (days)\ of\ mice\ in\ a\ group}{Total\ numbers\ of\ mice\ in\ that\ group}$$

Body weight loss is one feature of malaria infections in mice. The body weight of each mouse was measured to determine the effectiveness of the extract. The body weight of each mouse in all the groups was taken before infection (day 0) and on day 4. Each mouse in a group was measured using a digital balance. Then, the average percent change in body weight was compared with that of the control groups.

Cytotoxicity Test on Peripheral Blood Mononuclear Cells (PBMCs)

The peripheral blood mononuclear cells (PBMCs) were extracted as previously described.[12]chloroform, ethyl acetate, acetone and methanol solvents by sequential maceration method and the extract was filtered mass was obtained at low room temperature under pressure in a rotary vaccum evaporator. The PBMC cells were cultured in 96-well plates containing RPMI-1640 medium. To that, various volumes of extracts were added to each well to achieve concentrations of extracts of 15, 60, 240 and 1000µg/ml, and then incubated for 48 hours. The metabolic activity of the cells was determined by the MTT assay. The MTT dye solution was added, and the plates were incubated at 37°C for 4 hours. The absorbance was measured at 570 nm and refence at reference at 650 nm. A comparison between treated and untreated cells was made.

Brine Shrimp Lethality Test

Brine shrimp lethality is an acute toxicity assay of plant extract and others based on the ability to kill a laboratory-cultured larva of *Artemia salina*. Solutions of the extracts were made in dimethyl sulphoxide (DMSO) at varying concentrations, and incubated in duplicate vials with 10 brine shrimp larvae in a total volume of 5 ml. Brine shrimp larvae incubated in vials with a mixture of DMSO (30 µl) and artificial seawater (3.8 g sea salt/l distilled water) served as a negative control. The nauplii were exposed to different concentrations of plant extract for 24 hours. After 24 h, the nauplii were examined against a lighted background, with a magnifying glass and the average number of surviving larvae was determined. The

percentage mortality was plotted against log concentration for assessment of the toxicity level of the extract.[13]

Oral Acute Toxicity Test

The acute toxicity study followed the Organisation for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals, specifically the "Up and Down" method (OECD No. 425), starting with the maximum permissible dose of 2000 mg/kg.[14] Female and male mice were acclimatised to the laboratory conditions for 5 days at 22ºC ± 1 and a 12-light/dark cycle. Before the experiment, the mice were fasted but allowed to water only for 3 hours and then randomly assigned into different groups each with six mice (3 females and 3 males). Groups 1 received 10% Tween 80 (5 mL/kg body weight) and group 2 received leaf crude extract (2000 mg/kg body weight). Female and male mice were kept in separate cages during the experiment period. The extracts and 10% Tween 80 were administered orally using an oral gavage. The animals were observed continuously for 1 hour for the presence of clinical signs of toxicity, then twice daily for 14 days. Body weight was recorded at day 0 before administration of the extract and before sacrifice at day 14. After dosing, the animals were observed individually at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. The animals were observed for clinical signs of toxicity, including changes in the skin and fur, eyes and mucous membranes, as well as alterations in the respiratory, circulatory, autonomic, and central nervous systems, and in somatomotor activity and behavioural patterns. Particular attention was given to the occurrence of tremors, convulsions, salivation, diarrhoea, lethargy, sleep, and coma.

Data Analysis

Data were analysed by GraphPad Prism software version 9.0. All comparisons among groups were done by one-way analysis of variance (ANOVA) and Dunnett's post hoc comparison test. Student t-test was used to compare the means of two groups. *P* value of less than .05 was considered statistically significant.

Ethical Approval

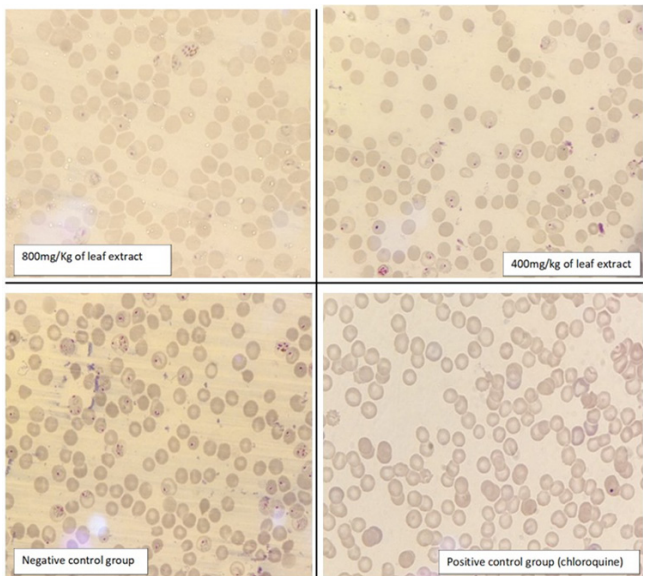
The ethical clearance was sought and obtained from Muhimbili University of Health and Allied Sciences (MUHAS) institutional review board with reference number; DA/25/111/01. The experimental animals were handled according to MUHAS animal care and use policy 2020 and international guidelines on the use of laboratory animals for research.

RESULTS

Antimalarial Activity of Materials Collected after the Heavy Rainfall Season

Oral administration of the hydroethanolic extracts of *Conyza pyrrhopappa* leaf extract in malaria-infected albino mice showed a dose-dependent antimalarial activity. On day four of the experiment, the highest percentage parasitaemia was observed in the negative control group, followed by the test group which received 400 mg/kg of the extract, and 800 mg/kg of the extract. No parasitaemia was observed in the positive control group, which received 10 mg/kg of chloroquine, Figure 1.

FIGURE 1: Photographic Presentation of Parasitemia in the Test and Control Groups on the Fourth Day of Treatment



The average percentage suppression in the test group which received 800 mg/kg body weight was 65.70%, while the percentage suppression in the test group which received 400 mg/kg body weight was 58.78%. The mean percentage parasitaemia in both groups which received the hydroethanolic extracts of the plant is statistically significantly different ($P<.05$) from the mean percentage parasitaemia in the negative control group. However, there was no statistically significant difference between the mean percentage parasitaemia between the two test groups, which received different doses of the hydroethanolic extracts of the plant, as shown in Table 1.

Antimalarial Activity of Plant Materials Collected after the Light Rainfall Season

The ethyl acetate leaf fraction had the highest and considerable in-vivo antimalarial activity with 39.7% parasite suppression, followed by crude root extract with 34.1% parasite suppression. The dichloromethane leaf fraction had the lowest activity of 8.54% parasite suppression.

Comparison of the mean survival time of each test group with the negative controls indicated that the mean survival time of the positive control was statistically significant compared with the negative control ($P<.001$), while the mean survival times of the other treatment groups were not statistically significant compared with the negative controls, Table 2.

All treatment groups, including the crude root extract at a dose of 400 mg/kg body weight, showed a statistically significant difference in mean percentage parasitaemia compared with the negative control group ($P<.05$), except the dichloromethane leaf fraction, Figure 2.

TABLE 1: Mean Percentage Parasitemia and Percentage Suppression in P. berghei ANKA-infected Mice Treated with *Conyza pyrrhopappa* Leaf Extract, with Comparison of Parasitemia Levels among Treatment Groups

Treatment Group	Dose (mg/kg)	Mean % Parasitemia (Mean ± SD, n=6)	% Suppression	Comparison among groups	Mean Difference	*P-value*
Negative Control	—	42.70 ± 14.42	0	vs 400 mg/kg vs 800 mg/kg vs Positive Control	25.07 28.05 42.70	.001 <.0001 <.0001
Extract Treatment	400	17.63 ± 8.90	58.8	vs 800 mg/kg vs Positive Control	2.98 17.63	.936 .019
Extract Treatment	800	14.65 ± 3.39	65.7	vs Positive Control	14.65	.059
Positive Control (Chloroquine)	10	0.00 ± 0.00	100	—	—	—

TABLE 2: In-vivo Antimalarial Activity of *Conyza pyrrhopappa* Leaf and Root Extracts at 400 mg/kg/day against Plasmodium Berghei ANKA

Treatment group	Mean percentage parasitemia (Mean±SD) n= 6	Mean percentage suppression of parasitemia at day 4	Mean survival time ±SD (days)
Negative control group (10% Tween 80)	55.14±7.928	0	5.57±1.272
Dichloromethane leaf fraction(400mg/kg)	50.43±5.701	8.54	5.66±1.751
Ethyl acetate leaf fraction(400mg/kg)	33.25±6.5	39.69	5.8±1.304
Crude leaf extract (400mg/kg)	42.25±9.586	23.37	6.16±2.858
Crude Root extract (400mg/kg)	36.36±3.943	34.06	5.00± 0.7071
Positive control group (CQ 10 mg/kg)	0.0±0.00	100	14.57±2.309***

***represents statistical significance Vs negative control ($P \leq .0001$)

Acute Toxicity

At a dose of 2000 mg/kg body weight of *Conyza pyrrhopappa* extract, no mortality was observed. The body weights of test animals of both the control and the treated groups showed no statistically significant difference ($P>.05$) as shown in Table 3. Behavioral observations of the test animals after dosing did not indicate any clinical signs of toxicity. Throughout the experimental period, the animals were monitored for potential toxic effects, including changes in the skin and fur, eyes and mucous membranes, as well as alterations in behavioural parameters; however, no observable adverse effects or signs of toxicity were detected.

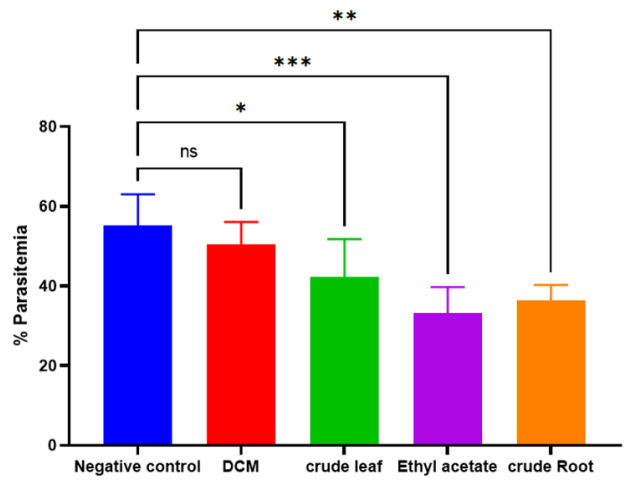
Brine Shrimp Lethality Test

Brine shrimp lethality test of *Conyza pyrrhopappa* hydroethanolic leaf extract showed LC_{50} (95%CI) value of 144.2 (110.9-187.5) µg/ml.

Cytotoxicity on PBMCs

Cytotoxicity activity of *Conyza pyrrhopappa* leaf extract was carried out against PBMCs at different concentrations to determine the IC_{50} by the MTT assay method. *Conyza pyrrhopappa* extract showed a significant effect on PBMCs in different concentrations with an IC_{50} (95% CI) of 94.56 (45.82–196.8) µg/ml, Figure 3.

FIGURE 2: Effect of *Conyza pyrrhopappa* on Percentage Parasitemia in the Test Groups and Control Groups



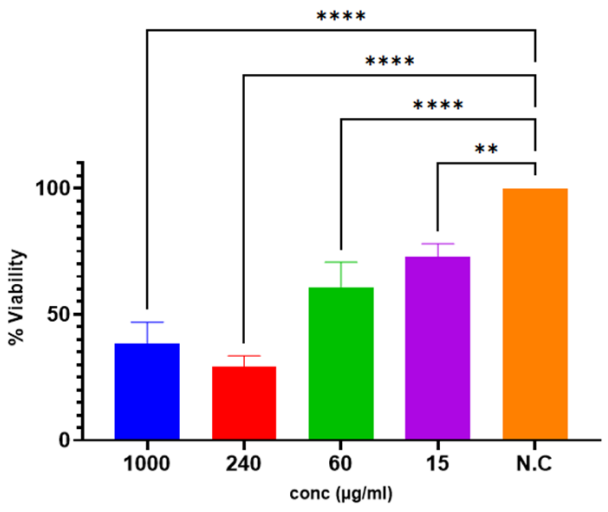
*Represents statistical significance vs negative control ($P \leq .05$).
**represents statistical significance vs negative control ($P \leq .01$).
***represents statistical significance vs negative control ($P \leq .001$).
Abbreviation: DCM, Dichloromethane .

TABLE 3: Effect of Single Dose of *Conyza Pyrrhopappa* Crude Leaf Extract on Body Weight of Mice

Group	*Body weight (g) Day 0	Day 7	Day 14	n	*P value*
Extract: 2000 mg/kg	27.20 ± 2.5	28.40 ± 3.5	29.40 ± 5.3	6	.69
Control vehicle (10% tween 80)	24.40 ± 2.1	24.60 ± 4.1	26.60 ± 9.6	6	.82

*Weight expressed in gram (g) and values are presented as Mean ± standard deviation (SD)

FIGURE 3: Cytotoxity of *Conyza pyrrhopappa* Crude Extracts on PBMCs at Different Concentrations



**Statistical significance against negative control ($P \leq .01$)
***Statistical significance against negative control ($P \leq .001$)
**** Statistical significance against negative control ($P \leq .0001$)
Abbreviations: N.C, Negative control; PBMCs, peripheral blood mononuclear cells.

DISCUSSION

This study reports the in-vivo antimalarial activity of extract and fractions of *Conyza pyrrhopappa* evaluated using the 4-day suppressive test. The leaf extract administered at a dose of 800 mg/kg body weight produced the highest mean parasitaemia suppression, achieving 65.7% inhibition. This result indicates a notable dose-dependent antimalarial effect of the plant extract in the experimental model. The method used evaluates the antimalarial activity of a candidate on early infections and has been used extensively in the discovery and development of conventional antimalarial drugs. The observed suppression in this study, therefore, suggests that *C. pyrrhopappa* contains bioactive constituents that may serve as potential leads for the development of novel antimalarial agents. Natural products have historically played a crucial role in antimalarial drug discovery, as demonstrated by quinine and artemisinin, indicating that plants with significant in-vivo activity remain valuable sources of new therapeutic compounds.

The study also revealed that the plant extract is not toxic. Furthermore, the results showed that the ethyl acetate leaf fraction exhibited the highest in-vivo parasite suppression compared with other extracts, suggesting that intermediate-polarity compounds present in this fraction may be responsible for the antimalarial properties of the leaves of *Conyza pyrrhopappa*. This observation is in agreement with several phytochemical studies indicating that many antimalarial compounds, including flavonoids, terpenoids, and other secondary metabolites, are frequently extracted in moderately polar solvents such as ethyl acetate.[7] Therefore, the enhancement of activity in this fraction supports the likelihood that specific bioactive molecules are concentrated within this polarity range.

In this study, plant materials collected just after the heavy rainy season demonstrated higher antimalarial activity than those collected just after the light rainy season. The observed difference in antimalarial activity may be attributed to seasonal variations in secondary metabolite concentrations, which are known to fluctuate in response to environmental stressors such as water availability, light intensity, and soil nutrient content.[15]

The crude root extract of *Conyza pyrrhopappa* exhibited higher in-vivo antimalarial activity compared with the crude leaf extract at the same dose of 400 mg/kg. This therefore suggests that it is important to further evaluate the antimalarial activity of the root, including the isolation of the active compounds. Roots of medicinal plants are often rich in defensive secondary metabolites that may possess strong pharmacological activities[7]. Therefore, the relatively higher activity observed in the root extract suggests that the root may represent a particularly promising source of antimalarial compounds for future bioassay-guided isolation studies.

The antimalarial activity of this plant is backed up by the reported efficacy of other plants of closely related species in the family of *Asteraceae*. *Conyza pyrrhopappa* is in the family of *Asteraceae* with many plant species reported to have activity against *Plasmodium falciparum*.[16] Different phytochemical compounds which pose antimalarial activity have been isolated from different parts of plants from this family. This plant has been reported elsewhere to contain clerodane diterpenes and flavonoids along with twelve known compounds.[17] The clerodane diterpenes has been found to possess pharmacological activities, including antitumor, antifungal, antibacterial, antipeptic ulcer, antiplasmodial, as well as hypoglycemic, hypolipidemic, and anti-thrombin inhibitory activity.[18] The antiplasmodial activity of clerodane diterpenes has been found in some plant including *Polyalthia longifolia*

(Sonn), in which bioassay guided fractionation of the extract yielded at three clerodane diterpenes and found to possess antimalarial activity,[19] and also activity found in *Laetia procera* (Poepp).[20] The presence of these bioactive classes of compounds in *C. pyrrhopappa* suggests that similar phytochemical constituents may contribute to the antimalarial activity observed in this study. Such compounds may interfere with parasite metabolism, heme detoxification, or redox balance within the parasite, mechanisms that are commonly targeted by existing antimalarial drugs. Therefore, further phytochemical investigation and bioassay-guided fractionation of this plant could lead to the identification of new antimalarial lead compounds.

With regard to safety assessment, the result of the acute toxicity test indicated no clinical signs of toxicity in mice. According to the globally harmonised classification system, chemicals are divided into five groups on their LD_{50} basis.[21] The results of this study suggest that the LD_{50} of the *Conyza pyrrhopappa* extract is above 2000 mg/kg. The hydroethanolic extract of *Conyza pyrrhopappa* leaf can be put in group 5 (LD50>2000 mg/kg), falling in the lower toxicity class and suggesting that the doses used in this study were safe. Low toxicity is an important consideration in antimalarial drug development because potential drug candidates must exhibit selective toxicity toward the parasite while maintaining minimal toxicity to host cells.

The brine shrimp lethality test is considered a rapid preliminary screening for the presence of biochemical activity and was used to determine the crude extract's toxicity. Extracts derived from natural products which have LC50≤100 µg/ml are known to possess toxicity according to a previously published article.[13] In this study, the LC_{50} value of hydroethanolic leaf extract of *Conyza pyrrhopappa* was 144.2µg/ml at 24 h, suggesting that it is not toxic. This further supports the safety profile of the extract and strengthens its potential for further pharmacological development.

The cytotoxicity testing was designed to detect the extent of damage caused by the hydroethanolic leaf extract of *Conyza pyrrhopappa* on normal human cells. The results indicate the extract has a low effect on PBMCs at lower doses. These observations are in accordance with the safety of other plants from the same family, including *Centaurea repens*, *Tanacetum parthenium* and *Ageratum conyzoides*.[21–24] Taken together, the antimalarial efficacy combined with low cytotoxicity could suggest a favourable therapeutic window, an important characteristic for candidate antimalarial agents.

In this study, fractionation was only performed on extracts collected just after the light rainy season, despite their lower antimalarial activity compared with extracts obtained from material collected just after the heavy rainy season. This was due to the limited amount of extract available. Future studies should therefore prioritize fractionation and phytochemical investigation of plant material collected just after heavy rainy season, as this period appears to yield extracts with stronger antimalarial activity. Such studies may facilitate the isolation and structural characterization of active compounds, which is a critical step toward the development of new antimalarial drugs from medicinal plants.

CONCLUSION

Conyza pyrrhopappa extracts demonstrated in-vivo antimalarial activity and safety. The ethyl acetate fraction of the leaf extract showed the most promising antimalarial activity, making it suitable for the isolation and identification of antimalarial active compounds. Acute toxicity, brine shrimp and in vitro cytotoxicity test suggested that *Conyza pyrrhopappa* extracts are not toxic. These findings support the traditional use of the leaves and roots in the treatment of malaria. Further phytochemical investigations on the ethyl acetate fraction of *Conyza pyrrhopappa* leaf extract to isolate, characterise, and identify the specific bioactive compounds responsible for the observed antimalarial activity are needed.

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