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Anti-onchocercal properties of three medicinal plants against *Onchocerca ochengi*

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ABSTRACT

Objective: To evaluate the filaricidal properties of three indigenous medicinal plants—*Cassia alata*, *Zornia scabra*, and *Momordica (M.) foetida*, in order to explore their potential as novel sources of filaricides for the sustainable management of filariasis.

Methods: Nine crude extracts from these three plants were prepared and screened *in vitro* against the microfilariae and adult worms of *Onchocerca (O.) ochengi*. Viability of *O. ochengi* adult male worm and mf was ascertained by microscopy while that of adult female worm of *O. ochengi* was by MTT/formazan assay. Cytotoxicity and acute toxicity were assessed in LLC-MK2 cells and in balb/c mice, respectively.

Results: At the single highest concentration of 500 µg/mL, all the extracts were active on *O. ochengi* adult males while seven showed activity on adult females and microfilariae. The methylene chloride extract of *M. foetida* was the most active on *O. ochengi* with IC_{50s} of 30.3 µg/mL, 40.3 µg/mL, and 44.2 µg/mL on males, females, and microfilariae, respectively. Selectivity indexes for the most active extract were 3.5 µg/mL (microfilariae), 3.9 µg/mL (females), and 5.1 µg/mL (males). There was no physical sign of acute toxicity and no hepatocellular injury to balb/c mice.

Conclusions: Extracts of *Cassia alata*, *Zheneria scabra* and *M. foetida* contain nematocidal properties with the methylene chloride extract of *M. foetida* being the most active on the adult worms and juvenile stages of *O. ochengi*. These findings provide a base for the isolation of anti-filarial lead molecules from the extract.

KEYWORDS: Onchocerciasis; Medicinal plants; *Momordica foetida*; Selectivity index

1. Introduction

Onchocerciasis, also known as river blindness, is caused by the filarial nematode *Onchocerca (O.) volvulus* and is responsible for disability, economic hardship, and social stigma in endemic areas[1]. The disease is transmitted through the bite of an infected

Significance

Current treatments for river blindness face challenges, including limited effectiveness against adult worms and concerns about ivermectin resistance. This study explores the filaricidal potential of three indigenous plants—*Cassia alata*, *Zornia scabra*, and *Momordica foetida*. Extracts, particularly from *Momordica foetida*, demonstrated strong activity against *Onchocerca ochengi* without cytotoxicity or acute toxicity in mice. These results support the development of new anti-filarial treatments and highlight the importance of local biodiversity and traditional knowledge in managing filariasis.

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blackfly (*Simulium damnosum*). It is a chronic debilitating disease characterized by troublesome itching, skin disfiguring and visual impairment including blindness. Of the estimated 20.9 people infected worldwide, more than 99% of the cases are in Africa[2]. The adult worms of this parasite can survive and reproduce for up to 14 years releasing hundreds to millions of microfilariae (mf). These mf can be ingested by the vector during a blood meal and subsequently develop into the infective larval stage to continue the life cycle[1].

Currently, there is no vaccine or macrofilaricide against onchocerciasis. Control of the infection since 1975 employed chemotherapy by repeated mass drug administration (MDA) of ivermectin (IVM) and doxycycline[3]. Community-directed treatment with ivermectin strategy is effective and sustainable. Despite the efforts of the various control programmes at eradicating the disease, it is unlikely that this disease will be eliminated by the target years[4]. Firstly, IVM is microfilaricidal only. Secondly, serious adverse events including encephalopathy and death have been reported in onchocerciasis-infected individuals who were co-infected with high microfilaraemia *Loa loa*[5]. Although doxycycline has been shown to exhibit some macrofilaricidal properties[6], it cannot be used in MDA because of the long duration of treatment (4-6 weeks) required[5]. Reports on suboptimal response to IVM[7–9] and the likelihood of emergence of resistance to IVM require the development of new therapies against onchocerciasis[10–12].

Herbal medications play a central role in the cure of several diseases, and since ancient times, plants have been used in traditional medicine[13]. Many medicinal plant-derived drugs have been isolated and delivered to the market. Additionally, the phenomenon of drug repurposing of natural products is promising[14–15]. *Cassia alata (C.)*, *Zheneria scabra (Z.)* and *Momordica (M.) foetida* aresome common plants routinely used in the local management of onchocerciasis and skin infections in Bambili, Northwest Cameroon. Bambili and its environs are characterized by green vegetation and fast-flowing streams which serve as good breeding sites for *Simulium damnosum*. These plants have shown activity against some parasites (*Plasmodium*, *Leishmania*) and are used to manage different ailments including skin infections, diarrhoea, fever, cough, and vomiting. Besides, they possess antioxidant, analgesic, anti-inflammatory, fungicidal and wound healing activity[16–22]. Despite their extensive use by traditional healers in treating skin diseases, the scientific assessment of their efficacy and safety is not ascertained. From empirical findings, we have reported their *in vitro* nematocidal effect against the adults and mf of *O. ochengi* and its safety in balb/c mice.

2. Materials and methods

2.1. Ethics approval

Ethical approval for all animal experiments was obtained from the Institutional Animal Care and Use Committee (UB-IACUC) referencing 2019/018/UB/IACUC/BTU/FS. The UB-IACUC reviewed and approved all experimental protocols, ensuring that they met the ethical standards and regulatory requirements for research using experimental animals. All personnel involved in the handling of animals and experimentation were trained in appropriate techniques to ensure the welfare of the animals. This test was conducted in accordance with the Organisation for Economic Co-operation and Development Guidelines for the Testing of Chemicals No. 420[23].

2.2. Collection and identification of plants

The leaves of three plants namely, *Z. scabra*, *C. alata* and *M. foetida* were collected based on ethnopharmacological information from Bambili, northwest region, Cameroon, during the rainy season. These plants were identified, and voucher specimens deposited at the National Herbarium in Yaounde, Cameroon with the following voucher numbers 33420/HNC, 66215/HNC, and 6815/SRF/CAM assigned for *M. foetida*, *C. alata* and *Z. scabra* respectively.

2.3. Preparation of crude extracts

The leaves of the plants were harvested, air-dried (3 weeks) and ground to fine powder using a well cleaned grinding mill. Each powder was weighed and then sequentially macerated with technical -grade hexane (H), methylene chloride (C) and methanol (M) for 48 h. The filtrate was then concentrated using a rotary evaporator (BUCHI Rotavapor R-200, Switzerland) and preserved at -20 ℃ . The yield (%) of each crude extract was determined using the following formula:

$$\text{Yield (\%)} = \frac{\text{Weight of crude extract}}{\text{Dry weight of plant part}} \times 100\%$$

A stock solution of 25 mg/mL was prepared by dissolving 25 mg of extract in 1 mL of sterile ⩾99.9% DMSO (SIGMA Life Science, USA) and stored at -200 ℃ until used in biological assays.

2.4. Phytochemical analysis of crude extracts

Phytochemicals such as alkaloids, flavanoids, steroids, saponins, cardiac glycosides, phenolics and tannins were determined using semi-quantitative standard methods as described elsewhere[24,25]. Mayer and freshly prepared Dragendoff's reagents were used for alkaloids, the Liebermann-Buchard test was employed for triterpenoids and sterols, $FeCl_3$ and $K_3Fe[(CN)_6]$, while Ferric chloride test was used for phenols, cardiac glycosides and tannins, while Cyanidine's test was for flavonoids and frothing test for saponins.

2.5. Biological assays

2.5.1. Isolation and culture of O. ochengi adult worms

Extraction of *O. ochengi* adult worms was done by method described by Cho-Ngwa *et al.*[26] with slight modifications. Briefly, fresh pieces of umbilical skin with palpable nodules were washed and sterilized. Nodules were carefully excised, and the recovered worm masses submerged in 2 mL complete culture medium (CCM) comprising RPMI-1640 supplemented with 25 mM HEPES, 2 g/L sodium bicarbonate, 0.3 g γ-irradiated L-glutamine powder, 5% heat inactivated newborn calf serum, 1X Antibiotic/Antimycotic solution; pH 7.4 in 12-well culture plates (NUNC, USA). The plates containing the worms were incubated overnight at 37 ℃ under an atmosphere of 5% CO_2 in humidified air in a (HERACELL-150i CO_2 incubator, USA). The viability of worms and sterility of cultures were evaluated using an inverted microscope (Nikon Eclipse TS100, China) before the addition of extracts.

2.5.2. Isolation of O. ochengi microfilariae

This was done using the skin sleeves method[27]. Fresh umbilical cattle skin was cleansed as above and shaved. Skin slivers were obtained and incubated for 2 h at room temperature in CCM. The emerged, highly motile mf were concentrated by centrifugation at 2000 rpm for 10 min. The microfilariae were re-suspended in CCM and distributed into each well (~ 15 mf/well) of a 96-well culture plate containing confluent monkey kidney epithelial cells (LLC-MK2). The cultures were kept at 37 ℃ under an atmosphere of 5% CO_2 humidified air for 24 h before adding extracts.

2.5.3. Preparation of mammalian cells

Monkey kidney epithelial cells (LLC-MK2) were purchased from the American Type Culture Collection (ATCC, Virginia, USA) and proliferated in CCM at 37 ℃ and 5% CO_2. Once the cells became fully confluent, the cell suspensions were distributed into 96-well culture plates. These viable cells served as feeder layers for the mf assays and cytotoxicity assessment of the extracts. The selectivity index (SI) of each extract was calculated as the ratio of the cytotoxic concentration (CC_{50}) of extracts on these mammalian cells to the inhibitory concentration (IC_{50}) of the extracts on the parasites[28].

2.5.4. In vitro screening of extracts on O. ochengi worms

The extracts were screened at 500 μg/mL in a primary screen and in triplicate for adult worms while mf was done in duplicate. Auranofin at 10 μM[29] served as positive control for the adult worm assay while amocarzine at 10 μM was used for the mf assay. Negative control wells contained only 2% DMSO (vehicle). The duration of the assay was 7 and 5 days for adult worms and mf respectively without any change in medium. All assays were conducted at 37 ℃ under an atmosphere of 5% CO_2. Adult male worm and mf viability was assessed using an inverted microscope[27]. Motility was scored daily, and the mean percentage motility inhibition scores attributed as follows: 100% (worms immotile), 75% (only head or tail of worm shaking, occasionally), 50% (whole body of worm motile but sluggish), 25% (almost vigorous motility), 0% (vigorous motility). Female worm viability was assessed using MTT/formazan assay[28,30]. The worms were incubated in 500 μL of 0.5 mg/mL MTT 3-(4,5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, from Sigma) for 30 min and the percentage of inhibition of formazan formation visually estimated. Activity-based scores assigned ranged from 100% parasite killing (no blue formazan coloration seen), 90% inhibition (few spots of blue color seen on a worm), 75% inhibition (about 75% of worm remained pale yellow), 50% (about 50% of worm remained pale yellow), to 25% (near total blue coloration), to 0% (entire worm appears blue as in negative control). An extract was considered active if there was ⩾ 90% mean inhibition of male worm motility, mf motility or formazan formation[31]. Active extracts were further re-tested in secondary assays at eight concentrations (500 to 3.91 μg/mL), to determine their IC_{50} values.

2.5.5. Cytotoxicity studies

Cytotoxicity was assessed using fully confluent LLCMK2 cells[29]. Briefly, active extracts were incubated with cells for 5 days and examined on the last day for deformities and detachment from the plate. Thereafter, the plate was decanted and 100 μL of 1 mg/mL MTT solution was added. After four hours of incubation, the solution was replaced with 200 μL of DMSO. The optical density

(OD) was read using an Emax precision microplate reader at 595 nm and the % inhibition of proliferation was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{OD of control - OD of treatment}}{\text{OD of control}} \times 100\%$$

The CC_{50} was calculated and used to determine the SI as follows:

$$SI = \frac{CC_{50} \text{ on LLCMK2}}{IC_{50} \text{ on parasite}}$$

2.5.6. Acute toxicity studies

This test was conducted in accordance with the Organisation for Economic Co-operation and Development Guidelines for the Testing of Chemicals[23] on the most active extract. Two groups of six nulliparous and non-pregnant female balb/c mice, about 10 weeks old (~ 20 g) were used. The test group was administered orally with a limited dose of 2 000 mg/kg body weight in a volume of 100 µL/10 g of body weight while the control group received the diluent only. The animals were observed daily for any changes in physical activity, food intake, water intake, stool sample, loss of fur, sensitivity to sound, motility and mortality for 14 days. On day 14, the animals were sacrificed, and levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using GOT(AST) and GPT(ALT) CHRONOLAB KIT (CHRONOLAB SYSTEMS S.L., Barcelona, Spain) as described by the manufacturer.

2.5.7. Data analyses

Results of the activity of the various extracts were analysed using GraphPad Prism software version 8.0 (GraphPad Prism Software, CA, USA), and expressed as mean ± SEM. IC_{50} values were calculated using a non-linear regression curve fit. The results of the phytochemical analyses were recorded categorically, indicating the presence and concentration of each phytochemical constituent.

3. Results

3.1. Activity of crude extract on *O. ochengi* worms

In total, nine crude extracts were obtained from three plants. At 500 µg/mL in the primary screen, all nine extracts inhibited adult males completely, while seven were active on adult females and mf. As shown in Table 1, the methylene chloride extract of Momordica foetida was the most potent on adult males (IC_{50s}=30.3 µg/mL), females (IC_{50s}=40.3 µg/mL) and mf (IC_{50s}=44.2 µg/mL).

3.2. Dose–dependent response of *O. ochengi* to plant crude extracts

Out of the six extracts that were re-tested in the secondary screen, the metanolic extract of *Z. scabra* (ZSM) and the methylene chloride extract of *M. foetida* (MFC) completely inhibited adult male motility (IC_{100}) at 62.5 µg/mL and only MFC showed complete inhibition of formazan at 62.5 µg/mL (Figure 1). Consistently, ZSM and MFC recorded the lowest IC_{100} of 125 µg/mL on mf in a time-dependent response manner. By the 24^{th} hour, all the active extracts completely inhibited motility at 500 µg/mL (Figure 2).

3.3. Toxicity studies

The IC_{50s} of the active extracts on *O. ochengi* and the selectivity indices showed that the most active extract equally recorded the

Table 1. Percentage yield, IC_{50}, and selectivity indexes of plant extracts on *O. ochengi* worms.

Plant name	Solvents	Extract code	Yield(%)	IC_{50} (µg/mL)			Selectivity indexes	
				O. ochengi male worm motility	Formazan formation by *O. ochengi* female worm	*O. ochengi* mf motility	adult female worms	adult male worms
Zheneria scabra	Hexane (H)	ZSH	0.9	118.5	255.1	95.5	0.6	0.3
	Methylene chloride (C)	ZSC	1.9	ND	inactive	ND	ND	ND
	Methanol (M)	ZSM	0.9	31.3	160.5	64.5	4.6	0.9
Cassia alata	Hexane (H)	CAH	2.5	79.7	303.5	Inactive	3.3	0.9
	Methylene chloride (C)	CAC	1.3	192.0	383.9	inactive	0.7	0.4
	Methanol (M)	CAM	0.8	ND	inactive	ND	ND	ND
Momordica foetida	Hexane (H)	MFH	0.5	95.8	258.0	121.1	2.0	0.4
	Methylene chloride (C)	MFC	7.2	30.3	40.3	44.2	5.1	3.9
	Methanol (M)	MFM	5.4	ND	ND	ND	ND	ND

ND: not done; mf: Microfilariae.

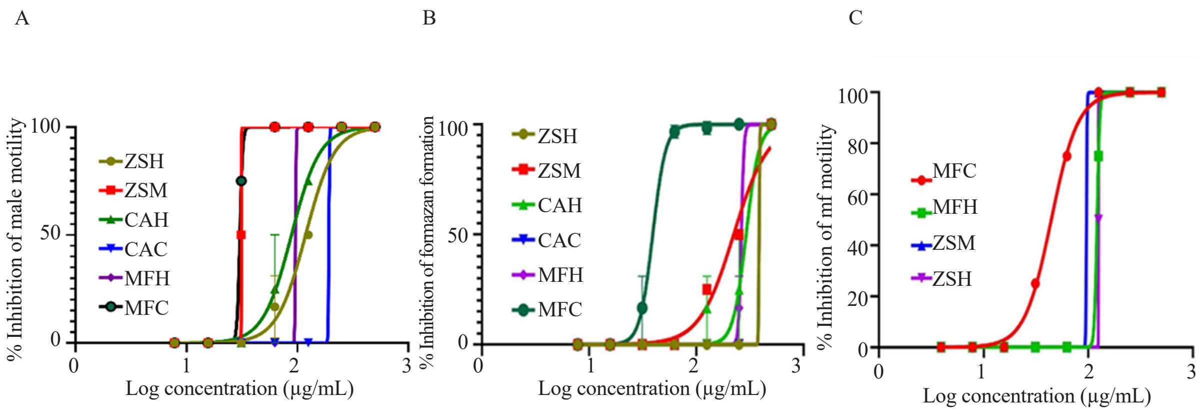

Figure 1. Dose-dependent response of *O. ochengi* to plant extracts after 7 days of culture (adult) and 5 days (microfilariae). Adult worms were tested in triplicate while mf were tested in duplicate. A: Adult males; B: Adult females and C: Microfilariae; ZSH: Hexane (H) of *Zheneria scabra*; ZSM: Methanol (M) of *Zheneria scabra*; CAH: Hexane (H) of *Cassia alata*; CAC: Methylene chloride (C) of *Cassia alata*; MFH: Hexane (H) of *Momordica foetida*; MFC: Methylene chloride (C) of *Momordica foetida*; $n = 3$.

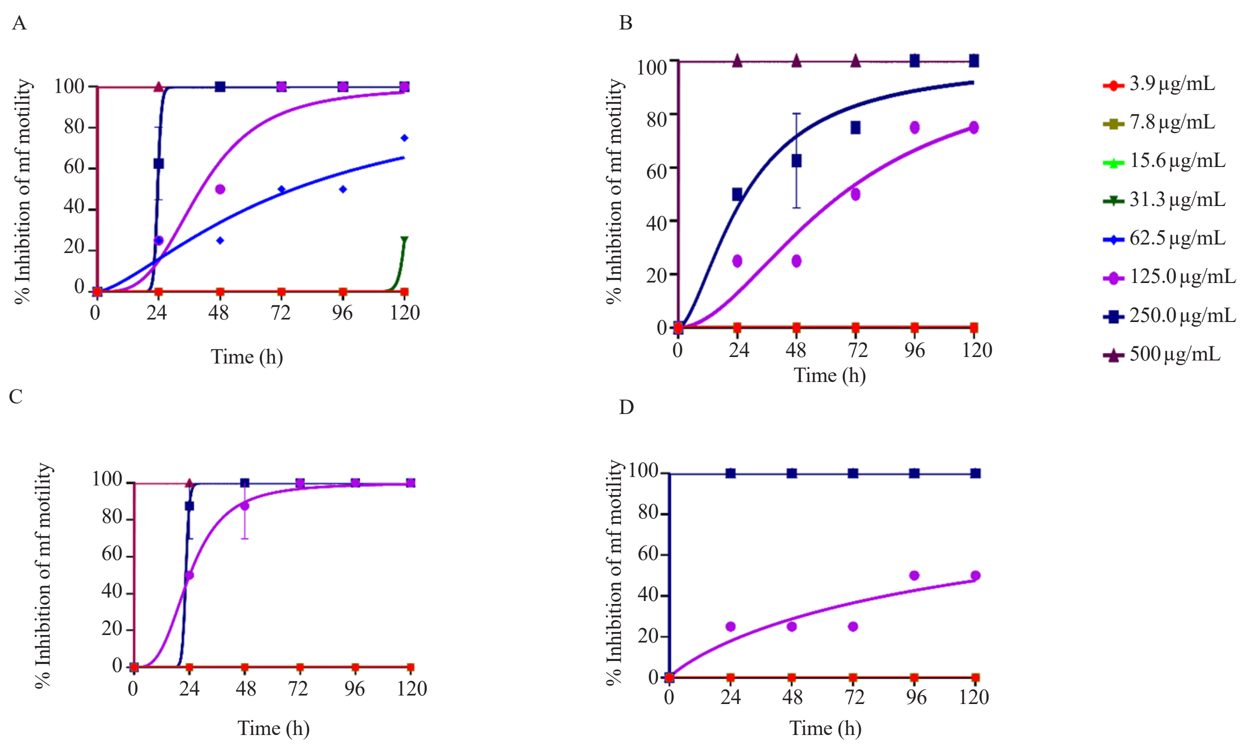

Figure 2. Time-dependent inhibition of *O. ochengi* microfilaria motility. Incubation with A) methylene chloride extract of *Momordica foetida*, B) hexane extract of *Momordica foetida*, C) methanol extract of *Zheneria scabra* and D) hexane extract of *Zheneria scabra*. $n = 3$.

Table 2. Phytochemical constituents of plant extracts.

Plant extract code	Class of compound						
	Steroids	Alkaloids	Cardiac glycosides	Phenolics	Tannins	Flavonoids	Saponins
ZSH	+++	-	+++	-	++	++	++
ZSC	+++	+	+++	-	+	+++	+++
ZSM	+	-	+++	+	+++	++	+++
CAH	+	-	++	+	_	++	+
CAC	+	-	++	-	_	+	++
CAM	+++	-	+	-	+++	+	+
MFH	+++	-	++	-	-	+	++
MFC	+++	-	+++	-	++	+	+
MFM	++	-	+++	+	+	+	+

Key: +: Low concentration; ++: Moderate concentration; +++: High concentration; -: Absent; ZS: Zheneria scabra; CA: Cassia alata; MF: Momordica foetida; H: Hexane; C: Methylene chloride; M: Methanol.

highest SI on all stages of the worms (Table 1). On balb/c mice, MFC showed no sign of acute toxicity. No physical change was observed in the physical appearance and behaviour of the mice pre- and post-treatment in both the test and control groups. The levels of ALT in the treatment (33.0±1.3 U/L) and the control groups (29.6±1.3 U/L) were all within the reference range (25-60 U/L). Similarly, AST levels in the treatment (65.4±2.3 U/L) and control groups (71.5±1.9 U/L) were within the normal range (50-100 U/L). There was no significant difference between the treatment groups and the control groups.

3.4. Phytochemical screening

Seven different phytochemical constituents were present. Steroids, cardiac glycosides, flavonoids and saponins were found in all the nine extracts screened while alkaloids and phenolics were present in one and three extracts respectively (Table 2).

4. Discussion

In this study, we investigated the filaricidal properties of nine crude extracts on different worm stages of *O. ochengi*. All the extracts were active on *O. ochengi* at the highest concentration of 500 µg/mL. The most active extract (MFC) exhibited a dose- and time-dependent potency with IC_{50s}<50 µg/mL against all the stages of *O. ochengi*. Selectivity indices of this extract ranged from 0.6 to 5.1 with no overt signs of acute toxicity and no hepatocellular injury in balb/c mice. For the first time, we have established the nematocidal effect of these plant extracts and validated their acclaimed activity in the local management of these diseases in endemic areas. We used *O. ochengi* for our onchocerciasis screening assay due to the difficulty and cost in obtaining enough quantity of *O. volvulus* parasite. This parasite is the closest relative to *O. volvulus*, thus serving as an ideal analogue in evaluating potential drugs against the disease[12,32].

Onchocerciasis is a neglected tropical disease that afflicts the impoverished and underprivileged in developing countries. Consequently, it is not on the priority list of pharmaceutical industries since the affected population lacks the economic resources to support a profitable drug market. Current drugs used in MDA are mainly free donations. These treatments notably, IVM, diethylcarbamazine, doxycycline and albendazole, used either singly or in combination have limitations. These include adverse effects, long treatment period, lack of macrofilaricidal properties and the emergence of resistance. These caveats jeopardize ongoing efforts to eradicate onchocerciasis thus underscoring the need to develop new therapies that can be used singly or in combination with existing tools to combat the infections.

More than 80% of the African population relies on herbal medication for the treatment of many diseases because of their effectiveness and availability[13]. The rich diversity of plant constituents and their secondary metabolites are important therapeutic agents and could also serve as starting materials to produce new drugs. Historically, pharmacologically active pure compounds have been produced from plants and natural products for the prevention and treatment of different diseases[33]. The leaves of *C. alata* is used in the treatment and local management of many diseases such as constipation, ringworm, intestinal parasitosis, syphilis, rash, and itching[34]. In veterinary medicine, the leaf decoctions of this plant are also used against external parasites such as mites and ticks. *Z. scabra* has been reported to be used in the treatment of skin diseases, worm infection and diarrhoea[35,36] while *M. foetida* is used in the treatment of diverse diseases including smallpox, boils, and snake bites[37].

In this study, we identified steroids, cardiac glycosides, flavonoid and saponins as the major phytochemical constituents present in these plants. These constituents can contribute to the pharmacological potential of plants by working singly or in synergy to kill the parasites. In different studies, some of these phytochemicals have been reported to possess antimicrobial, antiviral, and anti-parasitic properties and have shown antioxidant effects[38,39].

At the highest concentration of 500 µg/mL, all nine extracts were active on *O. ochengi* adult males, while seven showed activity on the adult females and mf. This suggests that these plants possess anti-onchocercal activity and could partly justify their use in the local treatment of onchocerciasis-associated pathologies. Several studies on the effect of plant extracts on *O. ochengi* have been reported, supporting the rich folk medicinal plants in Cameroon[25–27, 30, 40–43]. We observed a dose-dependent response of this non-polar extract which is consistent with reports on the nematocidal activity of non-polar compounds such as essential oils in other studies[30,40,43]. These promising results from non-polar extracts may provide more guidance in the search for new nematocides and phytomedicine. Our observation of the extract being more active in males of *O. ochengi* than females has been reported elsewhere on Cyperus articulates[30]. The difference in the order of potency against each stage and parasite species may be associated with different parasite morphology and pharmacological targets. The activity of MFC on these two parasites might reveal a broad spectrum of activity on nematodes and can support the phenomenon of drug repurposing of natural products.

An ideal filaricide should be safe. Our findings showed again that MFC was the safest as seen in the SI values. In mice, there was no observable sign of acute toxicity and no hepatocellular injury. This relatively low toxicity observed and supported by at least, the short-term absence of adverse effects in humans during traditional herbal treatment, further supports the use of the extract in the endemic areas as filaricidal remedies. This is an encouraging finding considering the number of efficacious drugs that have been abandoned because of safety issues. Further bioassay-guided fractionation and purification will hopefully unfold the active molecules for channelling into the drug discovery pipeline.

5. Conclusions

The methylene chloride extract of *M. foetida* is active against adult worms and juvenile stages of *O. ochengi* and safe in balb/c mice. These findings provide a base for the isolation of anti-filarial lead molecules from the extract.

Conflict of interest statement

The authors claim there is no conflict of interest.

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This study receives no extramural funding.

Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Samje M designed, performed the experiments, and drafted the manuscript, Ngoufo P collected plants, performed the experiments, and drafted the manuscript, Mfonku N, Tchoufack B, Mbah G and Tiku E performed experiments, Samje M, Mbah G and Ngoufo P analysed data, Cho-Ngwa F supervised and critically review the manuscript. All authors contributed to the final version of the manuscript.

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