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Exploring the antifilarial potential of an important medicinal plant *Typhonium trilobatum* (L. Schoot): Isolation, characterization, and structural elucidation of bioactive compounds against *Brugia malayi*

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ABSTRACT

Ethnopharmacology relevance: The plant *Typhonium trilobatum* has been utilized in traditional medicine for the treatment of many ailments, including parasitic infections. Recent examinations indicate that the bioactive substances from this plant may have antiparasitic activities against *Brugia malayi*, which have not been determined.

Purpose: The parasitic nematodes *Brugia malayi, Brugia timori,* and *Wuchereria bancrofti* causing lymphatic filariasis, remain a significant challenge to global public health. Given the ongoing nature of this enduring menace, the current research endeavours to examine the efficacy of an important medicinal plant, *Typhonium trilobatum*.

Methods: Different extracts of the *T. trilobatum* tubers were evaluated for their antiparasitic activity. The most prominent extract was subjected to Gas Chromatography Mass Spectrometry (GC-MS) and High Performance Liquid Chromatography (HPLC) followed by Column Chromatography for isolating bioactive molecules. The major compounds were isolated and characterized based on different spectroscopic techniques (FTIR, NMR and HRMS). Further, the antiparasitic activity of the isolated compounds was evaluated against *B. malayi* and compared with clinically used antifilarial drugs like Diethylcarbamazine and Ivermectin.

Results: The methanolic extract of the tuber exhibited significant antiparasitic activity compared to the other extracts. The bioactive molecules isolated from the crude extract were identified as Linoleic acid and Palmitic acid. Antiparasitic activity of both the compounds has been performed against *B. malayi* and compared with clinically used antifilarial drugs, Ivermectin and DEC. The IC_{50} value of Linoleic acid was found to be 6.09 ± 0.78 μg/ml after 24 h and 4.27 ± 0.63 μg/ml after 48 h, whereas for Palmitic acid the value was 12.35 ± 1.09 μg/ml after 24 h and 8.79 ± 0.94 μg/ml after 48 h. The IC_{50} values of both the molecules were found to be similar to the standard drug Ivermectin (IC_{50} value of 11.88 ± 1.07 μg/ml in 24 h and 2.74 ± 0.43 μg/ml in 48 h), and much better compared to the DEC (IC_{50} values of 194.2 ± 2.28 μg/ml in 24 h and 101.8 ± 2.06 μg/ml in 48 h). Furthermore, it has been observed that both the crude extracts and the isolated compounds do not exhibit any detrimental effects on the J774.A.1 macrophage cell line.

Conclusion: The isolation and characterization of bioactive compounds present in the methanolic tuber extract of *Typhonium trilobatum* were explored. Moreover, the antimicrofilarial activity of the crude extracts and its two major compounds were determined using *Brugia malayi* microfilarial parasites without any significant side effects.

1. Introduction

Lymphatic Filariasis (LF), is a neglected tropical disease caused by parasitic nematodes e.g. *Brugia malayi*, *Brugia timori,* and *Wuchereria bancrofti*. It is the second leading cause of permanent and long-term disability worldwide (Molyneux et al. 2003). It is one of the major health problems in 49 tropical and subtropical countries in Africa, Asia, South and Central America, and the Pacific Islands. According to the World Health Organization's (Lymphatic filariasis, 2022) global report,

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Abbreviations

GC-MS	Gas Chromatography Mass Spectrometry
HPLC	High Performance Liquid Chromatography
UV–Vis	Ultraviolet–visible spectroscopy
FTIR	Fourier transform infrared Spectroscopy
NMR	Nuclear Magnetic Resonance spectroscopy
HRMS	High-resolution mass spectrometry
LF	Lymphatic Filariasis
WHO	World Health Organization
DEC	Diethylcarbamazine
IVM	Ivermectin
ALB	Albendazole
MDA	Mass Drug Administration
mf	microfilariae
CO_2	carbon dioxide
FBS	Fetal bovine serum
DMSO	Dimethyl sulfoxide
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
IC_{50}	Half-maximal inhibitory concentration
CC_{50}	50% Cytotoxic concentration
SI	Selectivity Index
SD	Standard deviation
TTM	*Typhonium trilobatum* methanolic extract
TTE	*Typhonium trilobatum* ethanol extract
TTC	*Typhonium trilobatum* chloroform extract
TTEA	*Typhonium trilobatum* ethyl acetate extract
ANOVA	Analysis of variance formula
MA	Motility assay

Lymphatic filariasis still threatens 863 million people in 47 countries around the world. In 2018 nearly 51 million people were infected with LF as per the reports published by WHO. Moreover, India only contributes 40% of the global filariasis burden (Das et al., 2001). Despite recent additions to the antifilarial drugs such as Diethylcarbamazine (DEC), Ivermectin (IVM), and Albendazole (ALB) for the treatment of Lymphatic filariasis, only temporary microfilaricidal effect has been reported (Macfarlane et al., 2019). One reason for the poor success is that all three drugs, DEC, IVM, and ALB are microfilaricidal with apparently insignificant or no activity on adult parasites (Kalani et al., 2013; Debrah et al., 2011). As a result, peripheral blood microfilaremia reappears in patients after a certain period of withdrawal of the drug. Furthermore, the present drug of choice DEC, Ivermectin, and Albendazole are associated with adverse reactions that affect compliance with repeated treatment rounds (Cooper et al., 1999). The major side effects of Ivermectin are fever, pruritus, prostration, and postural hypotension (Awadzi et al., 1990, Chandler, 2018). Many of these adverse effects are characterized by progressive neurologic decline and encephalopathy within a few days of administration of the drug (Twum-Danso and loa, 2003; Chandler, 2018; Tagboto and Orish, 2022). There is evidence suggesting that extended and recurring administration of Ivermectin is linked to a higher occurrence of reactivity in allergen skin tests (Endara et al., 2010). With Albendazole, there have not been many reported cases of adverse effects due to its intake. However, incidences such as acute hepatitis in patients with continuous use of this drug have been reported (Amoruso et al., 2009). The implementation of Mass Drug Administration (MDA) programs for the widespread distribution of antifilarial medications has the potential to lead to the rapid development and spread of drug-resistant strains, ultimately undermining efforts to eradicate the infection (Cobo, 2016).

For the therapeutic management of LF, there is an urgent need for characterization of the new lead compounds that should kill both the microfilariae (mf) and adult worms (macrofilaricidal). Recently, several investigators reported many new potential antifilarial natural products isolated from plants or synthesized in the laboratory (Khunkitti et al., 2000; Lakshmi et al., 2004; Misra et al., 2007; Tripathi et al., 2003; Kushwaha et al., 2011, Das et al., 2015; Das, 2003, Kushwaha et al., 2016; Al-Abd et al., 2017; Lakshmi and Bhattacharya, 2018) that have both microfilaricidal and macrofilaricidal activity against rodent filariids (Tripathi et al., 2000).

Towards this end, we have recently identified a medicinal plant, *Typhonium trilobatum* (the tuber is used as a vegetable by the local communities) having an enormous therapeutic activity like antibacterial (Kandhasamy and Arunachalam, 2008; Saha et al., 2022), antifungal, anti-inflammatory, anti-diarrheal activities (Ali et al., 2012), anticancer (Khalivulla et al., 2019), antidiabetic (Manna et al., 2016). This prompted us to explore new lead molecules with promising antifilarial activity that do not cause undesired side effects (may be due to their edible properties). In this study, we are focusing on the antifilarial activity of this important medicinal plant and searching for the lead bioactive compound for which the plant possesses antifilarial activity. Herein, we have reported two major compounds of the isolated extract of the *T. trilobatum* plant. The compounds were identified and characterized based on their UV, FTIR, NMR, GC-MS, HPLC, and HRMS results. Subsequently, the isolated compounds were evaluated against *B. malayi* and compared with clinically used antifilarial drugs like Diethylcarbamazine and Ivermectin.

2. Materials and methods

2.1. Collection of plant material and extraction of phytochemicals

The tubers of the medicinal herb *Typhonium trilobatum* were collected from the Gandhamardhan hill located between Bargarh and Balangir District of Odisha, India. The plant was further identified and authenticated as *Typhonium trilobatum* L. Schotts by a Taxonomist and a voucher specimen (RPRC/11402) was deposited at the herbarium of the Regional Plant Resource Centre, Bhubaneswar. All procedures and plant material collection were conducted in accordance with local and national regulations and guidelines. The collected tubers were washed with running water to clean the soils or any earthy remains. These tubers were cut into small pieces, washed with water and dried in sunlight for the removal of water or moisture. The dried material was powdered in a pulverizer fitted with a mesh size of 1.5 mm. The powdered material was extracted out in different solvents like chloroform, ethyl acetate, ethanol, and methanol for 48 h by using the cold percolation method. The flowchart depicted in Fig. 1 demonstrates the sequential processes involved in the extraction, identification, and isolation of molecules. The different extracts were filtered using Whatman filter paper 41 and the solvents were evaporated using a rotary evaporator (Heidolph rotary evaporator, Germany). The dried extracts were tested for their *in vitro* antiparasitic activity against *B. malayi*.

2.2. GC-MS analysis of crude extract

The most effective crude extract was selected for GC-MS analysis (Fig. 2) after being tested *in vitro* for antiparasitic activity. GCMS analysis was done on Agilent 8890 coupled with Agilent 5977 MSD mass spectroscopy. Separation was carried out on an HP-5ms ultra inert capillary column (30 m × 250 μm x 0.25 μm). The chromatographic elution was programmed with an initial temperature of 75 °C and hold time of 0.5 min, then increased to 180 °C at a rate of 5 °C min^{-1} and hold time of 3 min, then increased to 300 °C at a rate of 5 °C min^{-1} and hold time of 5 min. The carrier gas was helium with a constant flow of 1.2 ml

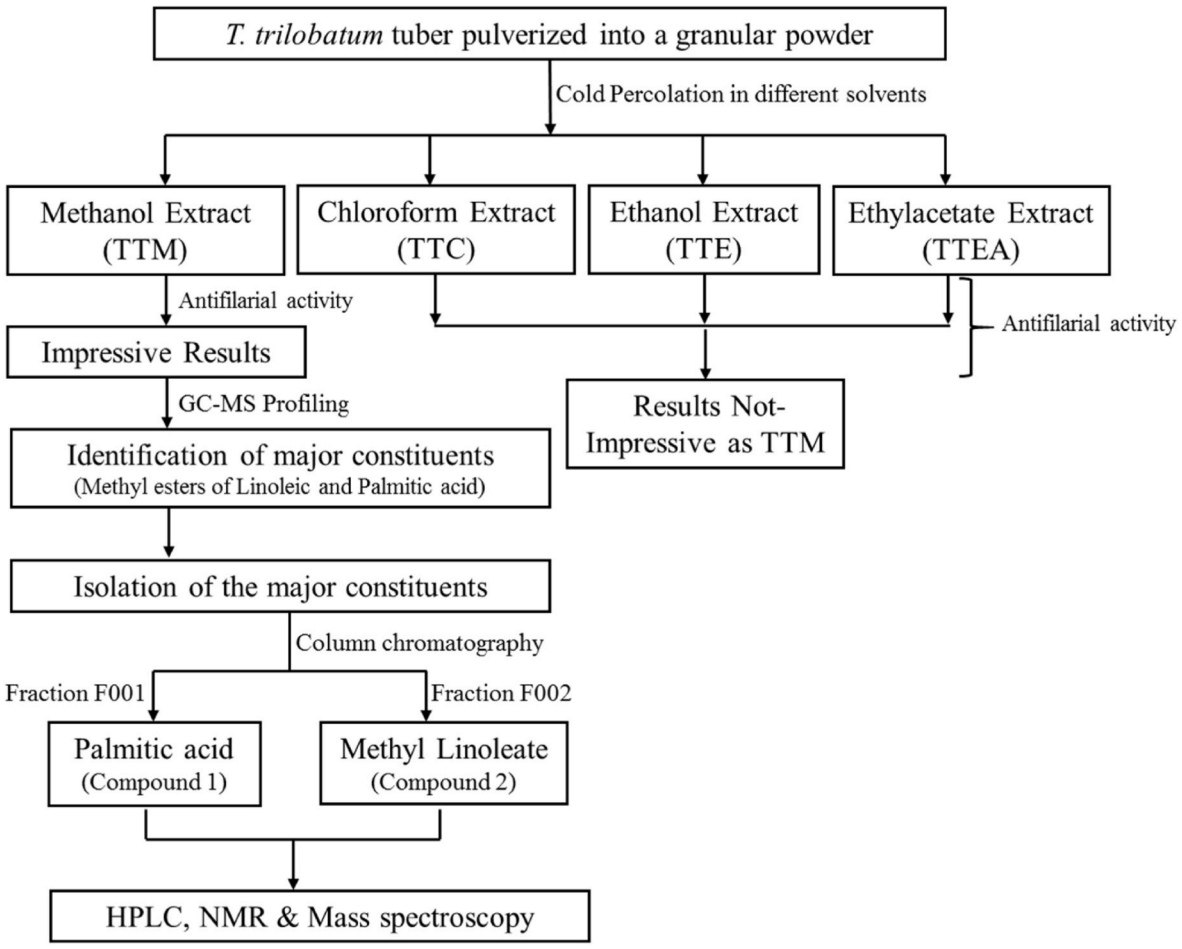

Fig. 1. Flowchart of extraction, identification, and isolation of bioactive compounds from *Typhonium trilobatum*. a. Cold percolation was performed for 48 h in different solvents followed by their antifilarial activity study. b. The antifilarial activity of Methanol extract (TTM) was superior to the other extracts and hence, selected for further studies. c. Identification of major compounds was performed by GC-MS on an HP-5ms capillary column with helium as carrier gas at a flow rate of 1.2 ml min^{-1} with a temperature gradient from 75 °C to 180 °C at a rate of 5 °C min^{-1} and a hold time of 3 min and from 180 °C to 300 °C at a rate of 5 °C and a hold time of 5 min. d. Palmitic acid (Compound 1) and Linoleic acid (Compound 2) were isolated by column chromatography using n-hexane: ethyl acetate (80: 20) and (70 : 30) respectively. e. Structural elucidation of the isolated compounds was confirmed by NMR and Mass spectroscopy whereas, molecular identification was done with HPLC against their reference substance.

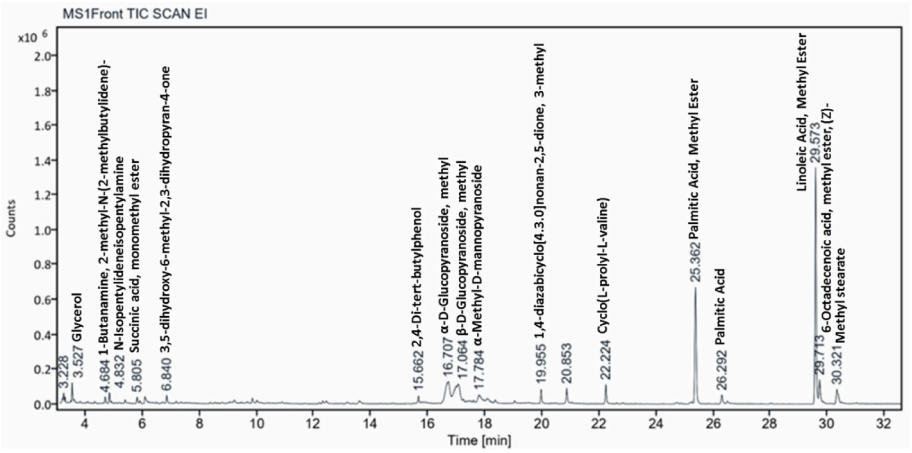

Fig. 2. GC-MS chromatogram of methanol extract of *Typhonium trilobatum* tubers. a. Phytochemical screening and identification of the chemical constituents in methanolic extract of *T. trilobatum* performed by GC-MS on an HP-5ms ultra inert capillary column with Helium as the carrier gas, flow rate of 1.2 ml min^{-1}, Temperature ramp from 75 °C to 180 °C at a rate of 5 °C min^{-1} and a hold time of 3 min and from 180 °C to 300 °C at a rate of 5 °C and a hold time of 5 min, inlet temperature: 250 °C and detector temperature: 250 °C. b. Mass transfer line maintained at a temperature of 280 °C and source at 230 °C. c. Processing of the chromatogram was done with Agilent MassHunter software and identification of the compounds was done with NIST 2020 database.

min^{-1}. The inlet temperature was set at 250 °C and the detector temperature was set at 250 °C. 1 μl of the sample (500 μg/ml) was injected in split mode with a split ratio of 15:1 and a solvent delay was opted for 3 min. For MS detection, ions were formed by electron impact at 230 °C using a mass-selective detector. The transfer line was maintained at 280 °C and masses were scanned in the quadrupole temperature of 150 °C from m/z 50 to 600 u. The obtained mass spectra were searched based on their percentage probability, score and reverse score for the identification of compounds by the NIST 2020 database (MS Search; NIST, MSS Ltd. Manchester, England).

2.3. Isolation characterization and structure elucidation of molecules

Following *in vitro* testing for antifilarial efficacy, the crude extract with the highest efficacy was chosen for further fractionation. Subsequently, the isolation of active components was performed by column chromatography over silica gel (60–120 mesh) using hexanes and ethyl acetate mixture as eluent. UV and IR spectra of isolated fractions were recorded with Shimadzu UV-2600i and Bruker Alpha II FTIR spectrophotometers respectively. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker AVANCE NEO (700 and 175 MHz, respectively) instruments internally referenced to tetramethylsilane (TMS) or DMSO signals. Chemical shifts were reported in parts per million and multiplicities are as indicated: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). Coupling constants, *J*, are reported in Hertz. Mass spectra were measured on UPLC-QTOF (Waters Acquity UPLC-I class plus) coupled with Waters XEVO G3 time of flight mass detector and photodiode array detector. All the available reagents and solvents were of superior grade and were used without further purification. Moreover, the compounds present in the crude extract were also confirmed through HPLC analysis (Waters alliance e2695 separation module with 2998 photodiode array detector).

2.4. Maintenance of microfilaria

The live microfilariae of *B. malayi* were transported by BEI Resource from the NIH/NIAID Filariasis Research Reagent Resource Centre (FR3; College of Veterinary Medicine, University of Georgia, U.S.A.). They were maintained in 75 cm^2 canted neck cell culture flasks in an incubator at 37 °C with 5% carbon dioxide (CO_2) containing 35 mL of RPMI-1640 supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Sigma-Aldrich) and amphotericin B (Sigma-Aldrich). The experiments were planned and carried out on the same day when *B. malayi* parasite was received.

2.5. In vitro antifilarial activity of T. trilobatum

2.5.1. Motility assay

The efficacy of the crude extract and isolated molecules on the viability of mf *in vitro* was assessed using motility assays (Murthy and Chatterjee, 1999). Test molecules were dissolved in DMSO and used at a concentration range of 5–500 μg/ml. Diethylcarbamazine (DEC) and Ivermectin (IVM) were used as reference compounds. DMSO ≤0.1% was used in place of the test agent solution as a control. The viability of the treated worms was assessed by calculating the percentage inhibition of the motility of microfilariae over DMSO-treated worms (Murthy and Chatterjee, 1999). Parasite motility was assessed under a microscope after 24 h and 48 h of exposure to the test substance. Loss of motility is defined as the inability of the worms to regain pre-treatment level motility even after incubating in fresh medium minus the test agent at 37 °C for 25–30 min and was expressed as a percentage (%) of control. IC_{50} value was determined as per earlier protocol with slight modifications (Huber and Koella, 1993) by using GraphPad v 9.5.0, Prism.

2.5.2. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for microfilaria viability

The MTT reduction method was used to evaluate the viability of microfilaria parasites (Comley et al. 1989; Taylor et al. 1996) with slight modifications. The microfilaria 5 × 10^4 numbers were seeded in a 96 well culture plate and the treatment was given at an optimized concentration of 100 μg/ml of the crude extracts, the isolated compounds and the standard drugs (DEC and IVM) each in triplicate and incubated in the CO_2 incubator at 37 °C, 5% CO_2 for 48 h. The microfilaria in ≤0.1% DMSO and complete media were considered as positive control. After incubation, the plate was centrifuged and the media was discarded. The pellet was resuspended in 10% MTT solution and incubated for 4 h at 37 °C. Then the 96 well culture plate was centrifuged with a 96 well plate cell wash centrifuge, washed with PBS, and resuspended with 200 μl of Dimethyl sulphoxide (DMSO). After 1 h of incubation, the absorbance was measured at 490 nm and the viability of microfilaria was assessed by the following formula.

$$\%\text{Viability} = \frac{(Absorbance\ of\ the\ test\ sample) - (Absorbance\ of\ diluent)}{(Absorbance\ of\ positive\ control) - (Absorbance\ of\ diluent)} \times 100$$

2.6. Determination of in vitro cytotoxic effect of the extracts and isolated compounds

The cytotoxicity test of the crude extracts and the isolated compounds was conducted using the methodof Page et al. (1993) with slight modifications (Nakayama, 1997). Briefly, adherent macrophage cells (J774.A.1) (5 × 10^3 cells/well) were grown in 96-well tissue culture plates and exposed to the crude extracts and the isolated molecules along with reference drugs DEC, IVM (12.5–800 μg/ml) for 48 h. Plates with a final volume of 100 μl per well were incubated in 5% CO_2 atmosphere at 37 °C. After 48 h of incubation, 10% of Alamar blue as a viability indicator was introduced. Further, after 4 h of incubation, the plates were read in a plate reader (iMark, Biorad) at 570 nm and 600 nm (reference wavelength) and expressed as a percentage of the cells in the control after background absorbance was subtracted. The CC_{50} was estimated using non-linear regression (Adams et al., 2020). In each of the two independent test, triplicates of the assay were conducted.

The Selectivity Index (SI) of the test samples was computed by the formula SI=CC_{50}/IC_{50}.

2.7. Statistical analysis

The values were expressed as mean ± standard deviation (SD) and statistical significance in relation to the control group was determined at a significance level of $p < 0.05$. The data obtained in this research were subjected to analysis through the utilization of one-way and two-way ANOVA, which was subsequently, followed by a posthoc Tukey Kramer multiple comparison test, utilizing Graph Pad Prism software. 9.5.1.

3. Results

3.1. The chemical composition of crude methanol extract was investigated by GC-MS

Gas chromatography-mass spectrometry (GC-MS) was used to investigate the chemical profile (Fig. 2) of the crude methanol extract of *T. trilobatum*. Sixteen compounds were identified with an area percentage of 96.23%, with two major peaks having retention times of 25.36 min and 29.57 min. These compounds were identified as Palmitic acid methyl ester (22.67%) and Linoleic acid methyl ester (40.53%) respectively. Likewise, fourteen more compounds were detected i.e. Glycerol, 1-Butanamine, 2-, methyl-N-(2-methylbutylidene), N-Isopentylideneisopentylamine, Succinic acid monomethylester,3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-

one,2,4-Di-tertbutylphenol, α-D-Glucopyranoside methyl, β-D-Glucopyranoside methyl, α-Methyl-D-mannopyranoside, 1,4-diazabicyclo[4.3.0] nonan-2,5-dione, 3-methyl, Cyclo(L-prolyl-L-valine, 6-Octadecenoic acid methyl ester, (Z)-, Methyl stearate. The compounds that have been identified, together with their respective molecular mass, retention time, and concentration (expressed as peak area percentage), are included in Table 1.

3.2. Isolation, characterization, and structural elucidation of the two major compounds isolated from the methanolic crude extract

Isolation of the major compounds in the crude methanolic extract of *T. trilobatum* tubers was carried out using column chromatography. Several fractions were collected in which one major compound was present in Fraction 001 and another major compound was present in Fraction 002. The two pure compounds were collected by drying the eluent in a rotary evaporator.The structure of Compound 1 (Fig. 3 A) and Compound 2 (Fig. 3 B) was shown in (Fig. 3). Characterizations of the compounds based on the UV, FT-IR, NMR and Mass spectroscopy are shown below.

Compound 1 (Palmitic acid) White; UV–Visible: 275 nm (n⟶ π* transition); FT-IR: ν_{max}/cm^{-1} 2923 (-CH_3), 2855 (-CH_2) 1743 (C═O); ^{1}H NMR (700 MHz, DMSO-d_6): δ 0.861 (3H, t), 1.253–1.413 (24H, m), 2.289–2.363 (2H, m), 2.753 (2H,t); 13C NMR (175 MHz, DMSO-d_6): δ 14.11, 22.70, 24.68,29.07, 29.24, 29.37, 29.44, 29.68, 31.93, 34.10, 180.38; LC-HRMS (m/z): $C_{16}H_{32}O_2$: 256.2723.

Compound 2 (Methyl Linoleate) Colourless Liquid; UV–Visible: 234 nm (n⟶ π* transition); FT-IR: ν_{max}/cm^{-1} 3009 (C═C–H), 2853 (-CH_2) 1704 (C═O); ^{1}H NMR (700 MHz, DMSO-d_6): δ 0.895 (terminal –CH_3, t), 1.202 (-CH_2 in fatty acid chain, m), 1.873 (-CH_2 in β-position w.r.t. carbonylic group, m), 1.988 (-CH_2 in both sides of olefinic hydrogen, m), 2.159 (-CH_2 in α-position w.r.t. carbonylic group, t), 2.689 (-CH_2 hydrogen of ester, s), 3.287 (-CH_2 between olefinic hydrogen, t), 4.014 (CH_2═CH_2 signals from the fatty acids, m); ^{13}C NMR (175 MHz, DMSO-d_6): δ 17.63, 20.85, 21.16, 22.77, 28.06, 29.41, 29.83, 30.53, 31.94, 34.79, 48.91, 127.03, 128.63, 129.08, 130.26, 174.26; LC-HRMS (m/z): $[M-H]^+$ for $C_{19}H_{34}O_2$: 293.1270.

Table 1
GC-MS analysis of crude methanol tuber extract of *Typhonium trilobatum*.

Sl No.	Retention Time (RT)	Compound Name	Molecular Mass	Area %
1	3.527	Glycerol	92.09	2.31
2	4.684	1-Butanamine, 2-methyl-N-(2-methylbutylidene)-	155.28	0.76
3	4.832	N-Isopentylideneisopentylamine	155.28	1.32
4	5.805	Succinic acid, monomethyl ester	132.11	0.69
5	6.840	3,5-dihydroxy-6-methyl-2,3-dihydropyran-4-one	144.12	0.80
6	15.662	2,4-Di-tert-butylphenol	355.32	0.77
7	16.707	α-D-Glucopyranoside, methyl	194.18	5.50
8	17.064	β-D-Glucopyranoside, methyl	194.18	3.34
9	17.784	α-Methyl-D-mannopyranoside	194.18	1.71
10	19.955	1,4-diazabicyclo[4.3.0]nonan-2,5-dione, 3-methyl	124.18	2.02
11	22.224	Cyclo(L-prolyl-L-valine)	196.25	3.34
12	25.362	Palmitic acid, methyl ester	270.45	22.67
13	26.292	Palmitic acid	256.43	1.85
14	29.573	Linoleic acid, methyl ester	294.47	40.53
15	29.713	6-Octadecenoic acid, methyl ester, (Z)-	296.49	4.41
16	30.321	Methyl stearate	298.50	4.21
		Total		**96.23**

a. GC-MS analysis of crude methanol tuber extract of *Typhonium trilobatum* was performed on an HP-5ms ultra inert capillary column with the carrier gas as Helium at a flow rate of 1.2 ml min^{-1}. The temperature ramping was from 75 °C to 180 °C at a rate of 5 °C min^{-1} and a hold time of 3 min and from 180 °C to 300 °C at a rate of 5 °C and a hold time of 5 min. The inlet temperature: 250 °C and detector temperature: 250 °C. b. MS transfer line maintained at a temperature of 280 °C and source at 230 °C. c. Processing of the chromatogram was done with Agilent MassHunter software and identification of the compounds was done with NIST 2020 database (MS Search; NIST, MSS Ltd. Manchester, England).

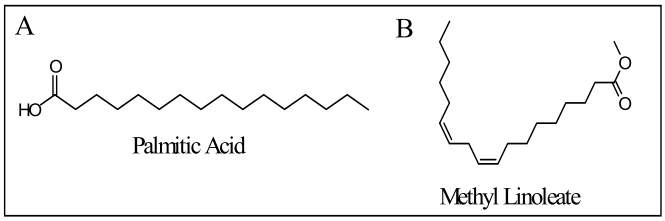

Fig. 3. Structure of the compounds isolated from the Methanol crude tuber extract of *Typhonium trilobatum*. A. Palmitic acid was isolated from the Fraction 001 after column chromatography using the eluent as n-hexane: ethyl acetate (80 : 20). B. Methyl Linoleate (Linoleic acid methyl ester was isolated from Fraction 002 by column chromatography using the eluent as n-hexane: ethyl acetate (70 : 30).

3.3. Confirmation of palmitic acid and linoleic acid by HPLC in the crude methanolic extract

The utilization of High-Performance Liquid Chromatography (HPLC) analysis was employed in order to identify the existence of Palmitic acid and Linoleic acid in the crude methanol tuber extract of *T. trilobatum*. The comparison of retention time between the analytes in the sample and standard solutions was conducted, and the identification of peaks was accomplished by means of different chromatograms (Fig. 4). The HPLC study of the crude extract demonstrated the existence of two prominent peaks of Palmitic acid and Linoleic acid (Fig. 4 A) in the sample solution at retention times of 22.84 min and 23.26 min, respectively. It aligned with the chromatograms of the standard solution of Palmitic acid at the retention time of 22.84 min (Fig. 4 B) and Linoleic acid at the retention time of 23.37 min (Fig. 4 C) confirming the presence of both of these compounds in the crude methanolic extract of *T. trilobatum*. The peak purity plots of Palmitic acid and Linoleic acid confirm the purity of both the peaks in the standard and sample chromatogram. The peak purity is determined by the purity angle and purity threshold where the peak angle is less than the purity threshold. It confirms that, there is no interference of any other peaks at the same retention time in the standard solution of palmitic acid and linoleic acid and in the crude sample solution.

3.4. T. trilobatum extracts and pure isolated compounds revealed antiparasitic activity in motility assay

In the current investigation, we examined the effects of a variety of solvent extracts of the *T. trilobatum* tuber such as chloroform, ethyl acetate, ethanol, and methanol. Also the compounds that were isolated from the methanol crude extract were evaluated. The microfilaricidal activity of the extracts and isolated compound on *B. malayi* microfilaria was assessed through an *in vitro* motility assay to determine its efficacy. The percentage motility of microfilaria cultured in the presence and absence of the plant extracts, isolated molecules, and standard drug Ivermectin (IVM) and Diethylcarbamazine (DEC) after 24 h (Fig. 5 A) and 48 h (Fig. 5 B) were compared in a dose-dependent manner (Fig. 5). From the experimental results shown in Table 2, it was revealed that the crude methanolic extract (TTM) had a more effective anti-microfilaricidal activity *in vitro*, with an IC_{50} value of 42.21 ± 1.81 μg/ml in 24 h and 32.73 ± 1.51 μg/ml in 48 h of treatment. For ethanolic extract (TTE), it was found that the IC_{50} value in 24 h was 114.9 ± 3.04 μg/ml and in 48 h was 87.21 ± 1.94 μg/ml of treatment. In the case of chloroform extract (TTC), the IC_{50} value was 195.0 ± 2.29 μg/ml and 63.60 ± 1.80 μg/ml in 24 and 48 h respectively. Also, in ethyl acetate

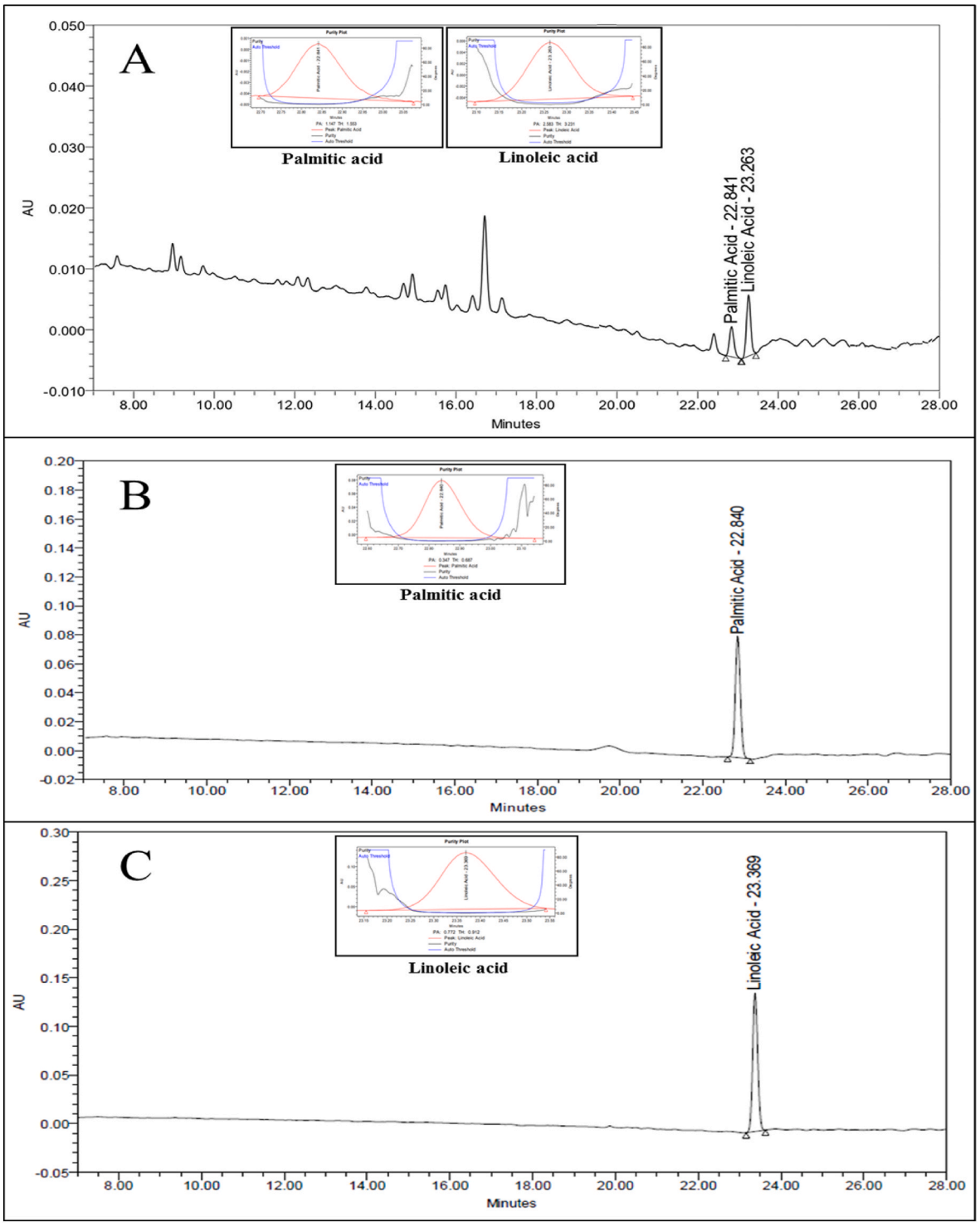

Fig. 4. HPLC analysis and peak purity plots for two major compounds Palmitic acid and Linoleic acid in methanol extract of *Typhonium trilobatum* tuber. (A) *Typhonium trilobatum* Crude extract (B) Standard Palmitic acid (C) Standard Linoleic acid. HPLC analysis was performed on a Waters X-Bridge C18 column at a flow rate of 1.0 ml min^{-1} with 0.1% formic acid in water as mobile phase A and 0.1% formic acid in acetonitrile as mobile phase B in a gradient mode with initial condition of 60% → 90% B from 0 → 20 min, 90% B from 20 → 25 min, 90% → 60% B from 25 → 25.1 min, 60% B from 25.1 → 35 min and detected at 210 nm. The column and sample oven temp was kept at 40 °C and 15 °C respectively.

(TTEA) extract, the IC_{50} value was found to be 77.79 ± 1.89 μg/ml in 24 h and 45.11 ± 1.65 μg/ml in 48 h. The isolated compounds from fraction F001 (Compound 1- Palmitic acid) inhibited the motility of microfilariae at different concentrations and had an IC_{50} value of 12.35 ± 1.09 μg/ml in 24 h and 8.79 ± 0.94 μg/ml in 48 h of drug exposure. Similarly, the most effective isolated compound from fraction F002 (Compound 2-Methyl Linoleate) had an IC_{50} value of 6.09 ± 0.78 μg/ml in 24 h and 4.27 ± 0.63 μg/ml in 48 h of drug exposure, whereas in the standard drug Ivermectin (IVM) the IC_{50} value was 11.88 ± 1.07 μg/ml in 24 h and 2.74 ± 0.43 μg/ml in 48 h of drug treatment. Also in Diethylcarbamazine , the IC_{50} value was 194.23 ± 2.88 in 24 h and 101.3 ± 2.01 in 48 h of drug exposure. In comparison to other extracts, it was observed

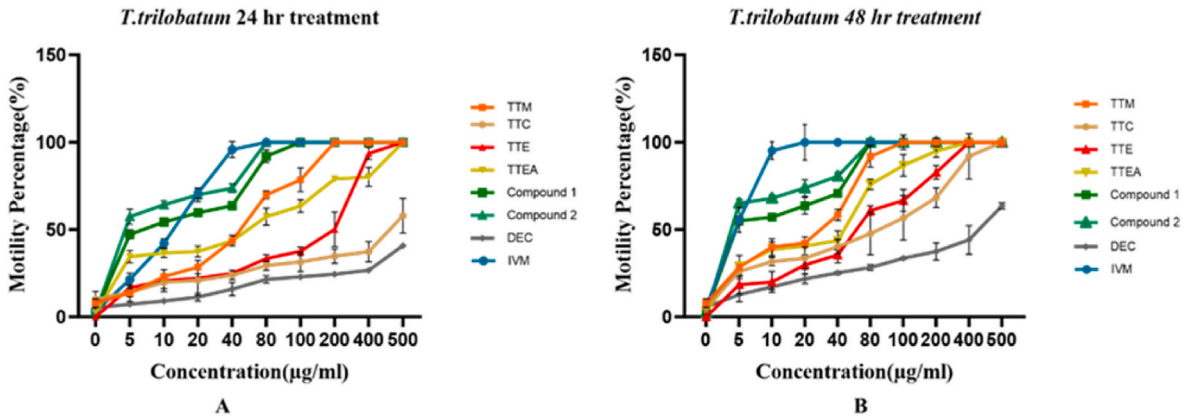

Fig. 5. Antifilarial activity of *T. trilobatum* tuber extracts and isolated compounds against *B. malayi* microfilaria parasite exposed to *T. trilobatum* methanol (TTM) extract, chloroform (TTC) extract, ethanol (TTE) extract, ethyl acetate (TTEA) extract, isolated compound 1, compound 2, Diethylcarbamazine (DEC), and Ivermectin (IVM) (A) 24 h of treatment (B) 48 h of treatment.

Table 2
Evaluation of *in vitro* efficacy of *T. trilobatum* crude extracts and its isolated compounds, as well as the standard drugs Ivermectin and Diethylcarbamazine (DEC), on microfilariae (mf) of *Brugia malayi* using motility assay (MA).

Antifilarial agent	[a]IC_{50} µg/ml (24 h)	[a]IC_{50} µg/ml (48 h)	[b]CC_{50} µg/ml	SI value (24 h)	SI value (48 h)
TTM	42.21 ± 1.81	32.73 ± 1.51	4236 ± 3.62	100.37	129.42
TTC	195.0 ± 2.29	63.60 ± 1.80	1215 ± 3.08	6.23	19.10
TTE	114.9 ± 3.04	87.21 ± 1.94	772.6 ± 2.88	6.72	8.85
TTEA	77.79 ± 1.89	45.11 ± 1.65	1557 ± 3.19	20.01	34.51
Compound 1	12.35 ± 1.09 (48.19 ± 4.25 µM)	8.791 ± 0.94 (34.30 ± 3.66 µM)	1017 ± 3.00	82.34	111.69
Compound 2	6.09 ± 0.78 (20.77 ± 2.66 µM)	4.27 ± 0.63 (14.56 ± 3.20 µM)	1218 ± 3.08	199.70	285.04
DEC	194.2 ± 2.28 (496.14 ± 5.82 µM)	101.8 ± 2.06 (260.08 ± 5.26 µM)	3227 ± 3.51	16.61	31.69
IVM	11.88 ± 1.07 (13.57 ± 1.22 µM)	2.74 ± 0.43 (3.13 ± 0.49 µM)	53.21 ± 1.72	4.47	19.41

Abbreviations: SI- Selectivity Index (CC_{50}/IC_{50}), TTM- *T. trilobatum* Methanol extract, TTC- *T. trilobatum* Chloroform extract, TTE- *T. trilobatum* Ethanol extract, TTEA- *T. trilobatum* Ethyl acetate extract, DEC- Diethylcarbamazine, IVM- Ivermectin.
[a] IC_{50} = concentration of the agent at which 50 % inhibition in motility of the parasites is achieved.
[b] CC_{50} = concentration at which 50 % of cells are killed.

that crude methanol extract and the isolated compounds from it exhibited greater efficacy in eradicating microfilaria. The isolated compounds exhibited higher levels of efficacy compared to the conventional drugs DEC and Ivermectin, which are commonly employed for treating filariasis.

The results of the two-way ANOVA indicated that there was no statistically significant difference in the mean of the dependent variables compared to the standard drug IVM, the crude methanol extract and the isolated molecules i, e. Palmitic acid and Methyl Linoleate ($p > 0.9999$). In comparison to the untreated control groups ($p < 0.0001$), the results were found to be highly significant, suggesting that the crude methanol extract and the isolated compounds (Palmitic acid and Methyl Linoleate) had a similar effect to that of the standard drug Ivermectin and had a significant impact on microfilaria. Furthermore, a notable interaction effect was detected between the two ways of drug treatment i,e. treatment in a dose-dependent way and time-dependent way. This indicates that the influence of the treatment methods and their relevant parameters has a significant difference across various degrees of treatment.

3.5. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay for microfilaria viability show antifilarial activity like standard drug Ivermectin

Microfilaria viability was dramatically decreased when compared to the control ($P < 0.001$), suggesting that *T. trilobatum* crude extracts and the isolated compounds have a time-dependent effect on microfilarial worm viability. However, the MTT assay showed a significant reduction of microfilaria after treating with *T. trilobatum* methanol crude extract (TTM), isolated Compound 1 (Palmitic acid) and Compound 2 (Methyl Linoleate) (Fig. 6). Also, *T. trilobatum* methanol extract (TTM), ethanol extract, ethyl acetate extract, chloroform extract, isolated Compound 1 (Palmitic acid), and Compound 2 (Methyl Linoleate) showed a strong microfilaricidal impact when compared to that of standard drug Diethylcarbamazine (DEC). It has been observed that, *B. malayi* microfilaria was killed when treated with TTM, isolated Compound 1 (Palmitic acid) and Compound 2 (Methyl Linoleate) after 24 h (Figs. 6 A) and 48 h of exposure (Fig. 6 B). There was no significant difference in the percentage viability of microfilaria treated with TTM, Compound 1, Compound 2 and those exposed to the conventional medicine Ivermectin. This finding prompted us to believe that the drug of choice, *T. trilobatum*, a medicinal plant, and the compounds that were isolated from this plant also possessed significant antifilarial efficacy compared to the gold-standard treatments.

3.6. Even at significantly higher concentrations, crude extracts and isolated compounds of T. trilobatum do not demonstrate any cytotoxic effects

The cytotoxicity test conducted after a 48 h drug exposure indicates that all the crude *T. trilobatum* extracts and the isolated compounds did not elicit any deleterious effects on the cells when treated at elevated concentrations (Fig. 7) as compared to the untreated control ($P < 0.0001$). In contrast, the non-linear regression analysis yielded a CC_{50} value of 4236 ± 3.62 µg/ml in methanol extract (TTM), 1215 ± 3.08 µg/ml in chloroform extract, 772.6 ± 2.88 µg/ml in ethanol extract and, 1557 ± 3.19 µg/ml in ethyl acetate extract of *T. trilobatum*. The CC_{50} value of 1017 ± 3.00 µg/ml and 1218 ± 3.08 µg/ml was observed in isolated Compound 1 (Palmitic acid) and Compound 2 (Methyl

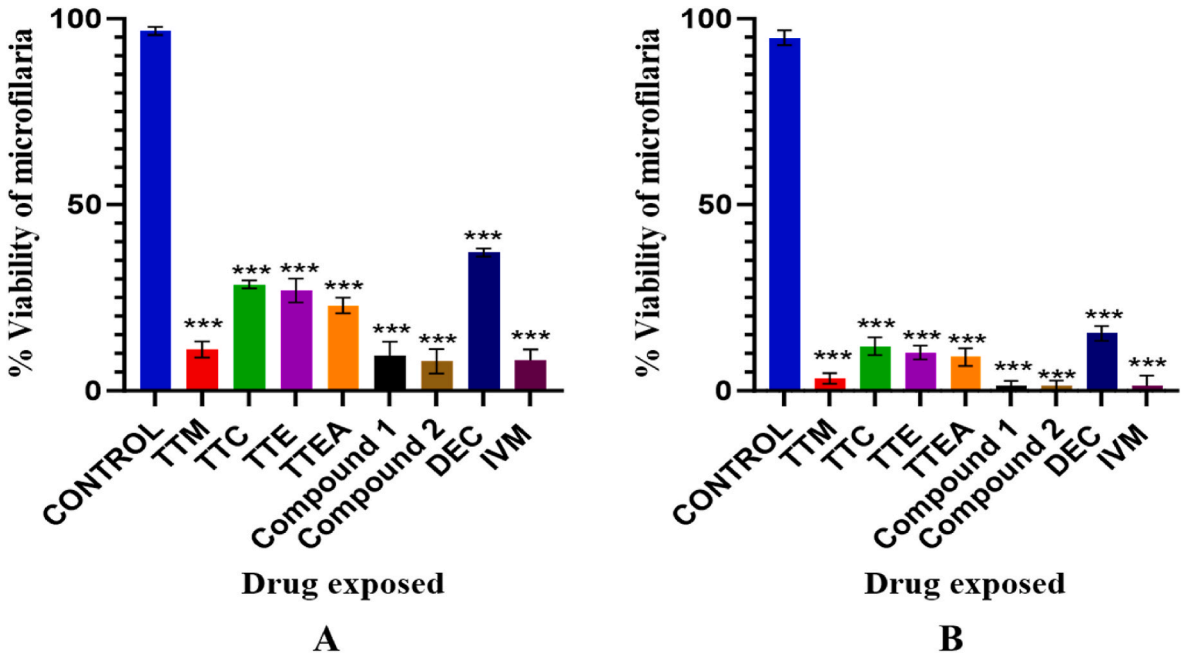

Fig. 6. The effect on *Brugia malayi* microfilariae viability (%+SD) as measured by MTT reduction (*B. malayi* was seeded in 96 well-cultured plates and treated with 100 μg/ml of crude extracts, compound 1, compound 2, standard DEC and standard IVM. It was incubated at 37 °C and 5% CO_2 for 24 and 48 h and MTT assay was conducted. The absorbance was measured at 490 nm and the percentage viability of microfilaria (mf) was calculated and expressed as a percentage of control values - as ***P < 0.001). (A) 24 h drug treatment (B) 48 h drug treatment.

Linoleate) respectively. The CC_{50} values of 3227 ± 3.50 μg/ml and 53.21 ± 1.72 μg/ml were observed in the standard drugs DEC and Ivermectin, respectively. This finding suggests the absence of any toxicity in the cell line. (Fig. 7, Table 2). The selectivity index (SI) of the crude extracts and the isolated compounds were observed to be higher as mentioned in Table 2 for the *Brugia malayi* microfilaria and in J774.A.1 murine macrophage cell line. In contrast, the SI value of the standard drugs in this particular cell line demonstrated a notable increase; this suggests that the crude extracts and the isolated compounds of *T. trilobatum* plant tuber did not exhibit cytotoxic properties.

4. Discussion

Typhonium trilobatum tuber is used in many Ayurveda formulations as a major component for the treatment of Lymphatic filariasis (P. Das, WO Pat., WO, 2011/145105 A3, 2011, Sangshetti et al., 2017). In this study, we validated this antifilarial claim of *T. trilobatum* by exploring the active components of this medicinal plant and those responsible for its antifilarial activity. Here, we performed an *in vitro* assessment of the *T. trilobatum* tuber extracts in different solvents and the isolated major compounds from the active extract for their antifilarial activity and cytotoxicity study. These isolated compounds were screened for their antifilarial activity against *B. malayi* microfilaria and compared with the standard drugs Ivermectin and Diethylcarbamazine. We performed the cytotoxicity study of the crude extracts and the isolated major compounds on the macrophage cell line (J774.A.1). The macrophage cell line (J774.A.1) was used in the toxicity study due to their characteristic features similar to human macrophage cells (Lemaire et al., 2014). Their phagocytic nature and ability to respond to various stimuli make them valuable for studying immune response and toxicity effects providing insights into potential hazards of substances on immune cells. We also described and elucidated the structure of the isolated bioactive molecules, by various spectroscopic techniques like UV, FT-IR, NMR, and mass spectrometry.

In the current investigation, it was observed that the methanol extract (TTM), revealed higher antiparasitic activity compared to other crude extracts i.e. ethyl acetate (TTEA), chloroform (TTC), and ethanol (TTE). Further, the isolated Compound 1 (Palmitic acid), and Compound 2 (Methyl Linoleate) exhibited significant activity compared to the crude extracts and the reference standard drug DEC and IVM.

In the crude methanolic extract, both Linoleic acid and Palmitic acid were present in high concentration, and both synergistically showed inhibitory action on microfilaria. As per the GC-MS data, the compound with the highest concentration, precisely 40.53%, (Table 1) was identified as the methyl ester of Linoleic acid, commonly referred to as Methyl Linoleate, which is a process derivative of Linoleic acid. Recent studies have consistently found that Linoleic acid is the predominant component exhibiting strong nematocidal activity (Panda et al., 2020). The presence of Linoleic acid, a potent bioactive compound, in the fruit *Holigarna caustic* showed strong nematocidal activity against *Caenorhabditis elegans* (*C. elegans*) with an IC_{50} value of 0.2 μg/ml. Our *in vitro* assessment reveals that the isolated compound Linoleic acid, methyl ester (Methyl linoleate) has a significant IC_{50} value against human filarial parasite *B. malayi* i.e. 6.09 ± 0.78 μg/ml in 24 h and 4.27 ± 0.63 μg/ml in 48 h (Fig. 5, Table 2). It also exhibited remarkable inhibition, surpassing the standard medication DEC and demonstrating comparable inhibition to IVM (Fig. 6), without any harmful side effects to the murine macrophage cell line J774.A.1 (CC_{50}: 1218 ± 3.08 μg/ml, SI: 285.04) even at high concentrations (Fig. 7).

Another major compound that was isolated from *T. trilobatum* methanolic extract is Palmitic acid, which demonstrated significant nematocidal activity and was also identified by GC-MS as Palmitic acid, methyl ester (22.67%, Table 1) which is a process derivative of Palmitic acid. Several studies have revealed that certain fatty acids, namely pentadecanoic acid, palmitic acid, palmitic acid methyl ester, β-sitosterol, stearic acid, and linoleic acid, exhibit nematocidal activity against *Haemonchus contortus*, a hematophagous gastrointestinal nematode and on *C. elegans* (Pineda-Alegría et al., 2020; Machado et al., 2015). In our investigation, the isolated Palmitic acid from the *T. trilobatum* methanolic extract showed remarkable antifilarial activity against the human filarial worm *B. malayi* with an IC_{50} value of 12.35 ± 1.09 μg/ml and 8.791 ± 0.94 μg/ml in 24 h and 48 h, respectively (Fig. 5, Table 1). Having no side effect to the macrophage cell line J774.A.1 (CC_{50}:1017 ± 3.00 μg/ml, SI: 111.69) which are shown characteristics of

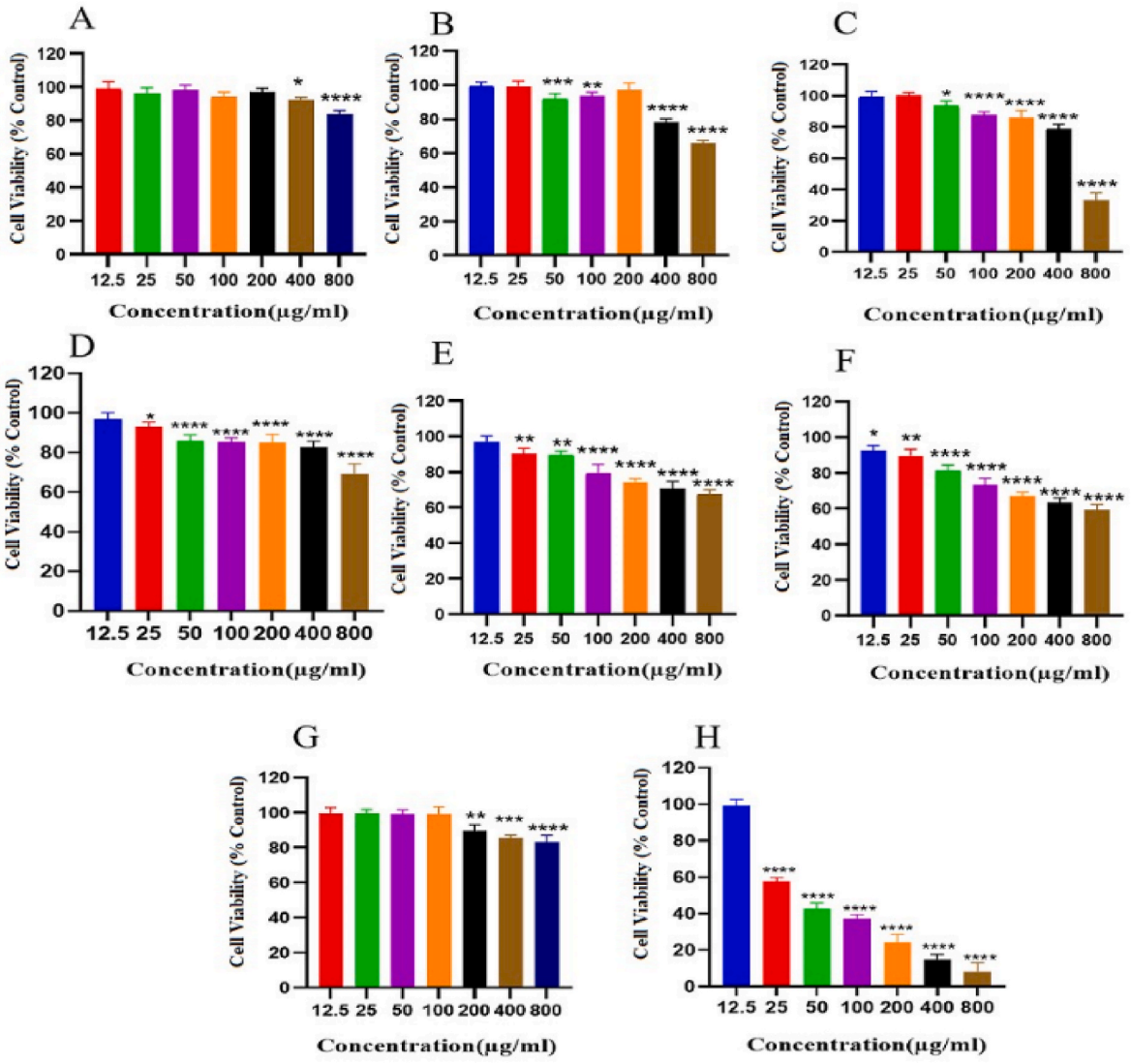

Fig. 7. Assessment of cytotoxicity after 48 h of treatment in a macrophage cell line (J774.A.1) for *T.trilobatum* (A) Methanol extract (TTM), (B) Chloroform extract (TTC), (C) Ethanol extract (TTE), (D) Ethyl acetate extract (TTEA), (E) Isolated compound 1, (F) Isolated compound 2 (G) Diethylcarbamazine (DEC), and (H) Ivermectin (IVM) and expressed as *P < 0.1,**P < 0.01, ***p < 0.001, ****P < 0.0001, and compared to control group having 100% viability.

macrophages found in the human body (Fig. 7).

Several plants and their isolated compounds (Kushwaha et al. 2019; Lakshmi et al., 2004; Misra et al., 2011) as well as synthetic derivatives (Sashidhara et al., 2014; Al-Abd et al., 2017) have been reported as antifilarial agents. In this study, we have successfully verified the existence of Linoleic acid and Palmitic acid in the crude methanol extract by gas chromatography-mass spectrometry (GC-MS) and high performance liquid chromatography (HPLC) analysis. HPLC analysis was carried out as an additional confirmation for the presence of Palmitic acid and Linoleic acid in the crude methanol extract using their respective reference standards. A purity plot for the peaks of Palmitic acid and Linoleic acid in standard and sample solutions were plotted which signifies no interference of any other peaks was observed at their specific retention time. Previously, it has been reported that fatty acids have significant activity against the nematodes. For an example, *Pleurotus djamor*, an edible mushroom contains β-sitosterol and various fatty acids which exhibit anthelmintic activity (Pineda-Alegría et al., 2017). Further, the standard drug Ivermectin showed cytotoxicity in the macrophage cell line (J774.A.1) when treated at a higher concentration while the crude extracts and the isolated compounds did not show any cytotoxicity when treated at the same concentration. As per the published scientific reports, Ivermectin showed cytotoxicity at a concentration greater than 100 µg/ml (Azeem et al., 2015).

The non-toxic effects of Linoleic acid and Palmitic acid on a macrophage cell line (J774.A.1), significantly comprehend the safety characteristics of these essential fatty acids under different experimental conditions. Linoleic acid, an omega-6 polyunsaturated fatty acid is an essential nutrient required for various cellular processes (Kaur et al., 2014, Djuricic and Calder, 2021) and Palmitic acid, a saturated fatty acid, has a crucial function in the formation and functionality of cell membranes, verifying the absence of toxicity in cell lines when exposed to moderate doses (Lima et al., 2002).

5. Conclusions

The identification, isolation and characterization of the major bioactive compounds present in the methanol tuber extract of *Typhonium trilobatum* was performed. Further, we have determined the antimicrofilarial activity of the crude extracts and the two isolated major compounds from methanolic extract against *Brugia malayi* microfilariae parasites. The crude extracts and the isolated compounds showed no signs of cytotoxicity in the *in vitro* cell line study. The antimicrofilarial

activity was found to be better compared to standard conventional drugs used in the treatment of lymphatic filariasis like DEC and Ivermectin. Since the standard drugs DEC and Ivermectin have some undesirable side effects, the methanolic extract of *T. trilobatum* tubers and the two bioactive compounds isolated from it could be preferred for the treatment of lymphatic filariasis. Overall we concluded that *Typhonium trilobatum* could be an useful resource for the exploration of different novel bioactive compounds that could be useful in targeting nematode parasites. Further, validation of the major compounds present in the tuber extract of this plant requires investigations into their mechanism of action, preclinical evaluation, and evaluation of their synergistic potential with the currently marketed anti-filarial medicines.

CRediT authorship contribution statement

Swaraj Kumar Babu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Dibya Ranjan Sahoo:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Prajna Parimita Mohanta:** Supervision, Methodology, Investigation. **Pradeep Kumar Naik:** Writing – review & editing, Validation, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2024.117858.

References

Adams, Y., Olsen, R.W., Bengtsson, A., Dalgaard, N., Zdioruk, M., Satpathi, S., Behera, P., Sahu, P.K., Lawler, S.E., Qvortrup, K., Wassmer, S.C., Jensen, A.T., 2020. P. falciparum erythrocyte membrane protein 1 variants induce cell swelling and disrupt the blood-brain-barrier in cerebral malaria. J. Exp. Med. 218 (3), e20201266, 2021.

Al-Abd, N.M., Nor, Z.M., Junaid, Q.O., Mansor, M., Hasan, M.S., Kassim, M., 2017. Antifilarial activity of caffeic acid phenethyl ester on *Brugia pahangi* in vitro and in vivo. Pathog. Glob. Health 111 (7), 388–394.

Ali, K., Ashraf, A., Biswas, N.N., 2012. Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhonium trilobatum* L. Schott. Asian Pac. J. Trop. Biomed. 2 (9), 722–726.

Amoruso, C., Fuoti, M., Miceli, V., Zito, E., Celano, M.R., De Giorgi, A., et al., 2009. Acute hepatitis as a side effect of albendazole a pediatric case. Pediatr. Med. e Chir. 31 (6), 262–264.

Awadzi, K., Dadzie, K.Y., De, Sole G., Remme, J., 1990. Reactions to ivermectin treatment in onchocerciasis patients. Acta Leiden. 59, 193—199.

Azeem, S., Ashraf, M., Rasheed, M.A., Anjum, A.A., Hameed, R., 2015. Evaluation of cytotoxicity and antiviral activity of ivermectin against Newcastle disease virus. Pak. J. Pharm. Sci. 28 (2).

Chandler, R.E., 2018. Serious neurological adverse events after ivermectin—do they occur beyond the indication of onchocerciasis. Am. J. Trop. Med. Hyg. 98 (2), 382.

Cobo, F., 2016. Determinants of parasite drug resistance in human lymphatic filariasis. Rev. Española Quimioter. 29.

Comley, J.C., Rees, M.J., Turner, C.H., Jenkins, D.C., 1989. Colorimetric quantitation of filarial viability. Int. J. Parasitol. 19 (1), 77–83.

Cooper, P.J., Awadzi, K., Ottesen, E.A., Remick, D., Nutman, T.B., 1999. Eosinophil sequestration and activation are associated with the onset and severity of systemic adverse reactions following the treatment of onchocerciasis with ivermectin. J. Infect. Dis. 179, 738—742.

Das, P., 2003. In vitro regeneration of *Typhonium trilobatum* Schott: a potential antifilarial plant via somatic embryogenesis. In: III World Congress on Medicinal and Aromatic Plants for Human Welfare. Chiang Mai, Thailand, February 3-7, 2003.

Das, P., 2011. Herbal Composition for the Treatment of Filariasis. WO Patent. WO 2011/145105 A3.

Das, P., Mohanty, S., Swain, D., Sahoo, P., Das, G., Naik, P.K., Hota, S.K., Bordoloi, M.J., Rao, HBD Prasad, 2015. *Typhonium trilobatum* (L.) Schott shows potency against lymphatic filariasis in man. Int. J. Indig. Med. Plants 48 (1), 1821. ISSN: 2051-4263.

Das, P.K., Ramaiah, K.D., Augustin, D.J., Ashok, Kumar, 2001. Towards elimination of lymphatic filariasis in India. Trends Parasitol. 17, 457–460.

Debrah, A.Y., Mand, S., Marfo-Debrekyei, Y., Batsa, L., Albers, A., Specht, S., Hoerauf, A., 2011. Macrofilaricidal activity in *Wuchereria bancrofti* after 2 weeks treatment with a combination of rifampicin plus doxycycline. J. Parasitol. Res. 2011, 1–9.

Djuricic, I., Calder, P.C., 2021. Beneficial outcomes of omega-6 and omega-3 polyunsaturated fatty acids on human health: an update for 2021. Nutrients 13 (7), 2421.

Endara, P., Vaca, M., Chico, M.E., Erazo, S., Oviedo, G., Quinzo, I., Cooper, P.J., 2010. Long term periodic anthelmintic treatments are associated with increased allergen skin reactivity. Clin. Exp. Allergy 40 (11), 1669–1677.

Kalani, K., Kushwaha, V., Verma, R., Murthy, P.K., Srivastava, S.K., 2013. Glycyrrhetinic acid and its analogs: a new class of antifilarial agents. Bioorg. Med. Chem. Lett 23, 2566–2570.

Kandhasamy, M., Arunachalam, K.D., 2008. Efficacy of *Typhonium trilobatum* (L.) Schott tuber extracts on pathogenic bacteria. Elect J Nat Subs 3, 1–7.

Kaur, N., Chugh, V., Gupta, A.K., 2014. Essential fatty acids as functional components of foods-a review. J. Food Sci. Technol. 51, 2289–2303.

Khalivulla, S.I., Mohammed, A., Sirajudeen, K.N., Shaik, M.I., Ye, W., Korivi, M., 2019. Novel phytochemical constituents and anticancer activities of the genus, typhonium. Curr. Drug Metab. 20 (12), 946–957.

Khunkitti, W., Fujimaki, Y., Aoki, Y., 2000. In vitro antifilarial activity of extracts of the medicinal plant *Cardiospermum halicacabum* against *Brugia pahangi*. J. Helminthol. 74, 241–246.

Kushwaha, V., Rastogi, S., Pandey, M.M., Saxena, K., Khatoon, S., Rawat, A.K., Murthy, P.K., 2019. In Vitro and in vivo antifilarial activity of standardized extract of *Calotropis procera* flowers against *Brugia malayi*. Curr. Top. Med. Chem. 19 (14), 1252–1262.

Kushwaha, V., Saxena, K., Verma, R., Verma, S.K., Katoch, D., Kumar, N., Singh, B., 2016. Antifilarial activity of diterpenoids from *Taxodium distichum*. Parasites Vectors 9 (1), 1–11.

Kushwaha, V., Saxena, K., Verma, S., Lakshmi, V., Sharma, R., Murthy, P., 2011. Antifilarial activity of gum from *Moringa oleifera* Lam. on human lymphatic filarial *Brugia malayi*. Chronicles Young Sci. 2 (4), 201-201.

Lakshmi, V., Bhattacharya, S.M., 2018. Antifilarial activity of *Gymnema sylvestre* R. Br Leaves against *Brugia malayi*. Biomed. J. Sci. Tech. Res. 7 (3), 5963–5966.

Lakshmi, V., Kumar, R., Gupta, P., Varshney, V., Srivastava, M.N., Dikshit, M., Murthy, P. K., Misra-Bhattacharya, S., 2004. The Antifilarial Activity of a Marine Red Alga.

Lemaire, S., Mingeot-Leclercq, M.P., Tulkens, P.M., Van Bambeke, F., 2014. Study of macrophage functions in murine J774 cells and human activated THP-1 cells exposed to oritavancin, a lipoglycopeptide with high cellular accumulation. Antimicrob. Agents Chemother. 58 (4), 2059–2066.

Lima, T.M., Kanunfre, C.C., Pompeia, C., Verlengia, R., Curi, R., 2002. Ranking the toxicity of fatty acids on Jurkat and Raji cells by flow cytometric analysis. Toxicol. Vitro 16 (6), 741–747.

Lymphatic filariasis, 2022. Keyfact. https://www.who.int/news-room/fact-sheets/detail/lymphatic-filariasis.

Macfarlane, C.L., Budhathoki, S.S., Johnson, S., Richardson, M., Garner, P., 2019. Albendazole alone or in combination with microfilaricidal drugs for lymphatic filariasis. Cochrane Database Syst. Rev. 1.

Machado, A.R.T., Ferreira, S.R., da Silva Medeiros, F., Fujiwara, R.T., de Souza Filho, J. D., Pimenta, L.P.S., 2015. Nematicidal activity of *Annona crassiflora* leaf extract on *Caenorhabditis elegans*. Parasites Vectors 8 (1), 1–5.

Manna, K., Debnath, B., Das, M., Marwein, S., 2016. A comprehensive review on pharmacognostical investigation and pharmacology of *Typhonium trilobatum*. J. Nat. Prod. 6 (3), 172–178.

Misra, N., Sharma, M., Raj, K., Dangi, A., Srivastava, S., Misra-Bhattacharya, S., 2007. Chemical constituents and antifilarial activity of *Lantana camara* against human lymphatic filariid *Brugia malayi* and rodent filariid *Acanthocheilonema viteae* maintained in rodent hosts. Parasitol. Res. 100, 439–448.

Misra, S., Verma, M., Mishra, S.K., Srivastava, S., Lakshmi, V., Misra-Bhattacharya, S., 2011. Gedunin and photogedunin of *Xylocarpus granatum* possess antifilarial activity against human lymphatic filarial parasite *Brugia malayi* in experimental rodent host. J Parasitol Res 109, 1351–1360.

Molyneux, D.H., Bradley, M., Hoerauf, A., Kyelem, D., Taylor, M.J., 2003. Mass drug treatment for lymphatic filariasis and onchocerciasis. Trends Parasitol. 19, 516–522.
Nakayama, G.R., 1997. Assessment of the Alamar Blue assay for cellular growth and viability in vitro. J. Immunol. Methods 204, 205–208.
Page, B., Page, M., Noel, C., 1993. A new fluorometric assay for cytotoxicity measurements in-vitro. Int. J. Oncol. 3 (3), 473–476.
Panda, S.K., Das, R., Mai, A.H., De Borggraeve, W.M., Luyten, W., 2020. Nematicidal activity of *Holigarna caustica* (Dennst.) oken fruit is due to linoleic acid. Biomol. 10 (7), 1043.
Pineda-Alegría, J.A., Sánchez, J.E., González-Cortazar, M., Von Son-de Fernex, E., González-Garduño, R., Mendoza-de Gives, P., Aguilar-Marcelino, L., 2020. In vitro nematocidal activity of commercial fatty acids and β-sitosterol against *Haemonchus contortus*. J. Helminthol. 94, e135.
Pineda-Alegría, J.A., Sánchez-Vázquez, J.E., Gonzalez-Cortazar, M., Zamilpa, A., López-Arellano, M.E., Cuevas-Padilla, E.J., Aguilar-Marcelino, L., 2017. The edible mushroom *Pleurotus djamor* produces metabolites with lethal activity against the parasitic nematode *Haemonchus contortus*. J. Med. Food 20 (12), 1184–1192.
Saha, P., Banerjee, A., Banerjee, G., Bag, P.K., 2022. Inhibitory activities of *Typhonium trilobatum* (L.) Schott on virulence potential of multi-drug resistant toxigenic Vibrio cholerae. Microb. Pathog. 165, 105485.
Sangshetti, J.N., Shinde, D.B., Kulkarni, A., Arote, R., 2017. Two decades of antifilarial drug discovery: a review. RSC Adv. 7 (33), 20628–20666.
Sashidhara, K.V., Rao, K.B., Kushwaha, V., Modukuri, R.K., Verma, R., Murthy, P.K., 2014. Synthesis and antifilarial activity of chalcone–thiazole derivatives against a human lymphatic filarial parasite, *Brugia malayi*. Eur. J. Med. Chem. 81, 473–480.
Tagboto, S., Orish, V., 2022. Drug development for onchocerciasis-the past, the present and the future. Front. trop. dis. 3, 953061.
Taylor, M.J., Cross, H.F., Mohammed, A.A., Trees, A.J., Bianco, A.E., 1996. Susceptibility of *Brugia malayi* and *Onchocerca lienalis* microfilariae to nitric oxide and hydrogen peroxide in cell-free culture and from IFN γ-activated macrophages. Parasitol. 112 (3), 315–322.
Tripathi, R.P., Tiwari, V.K., Bhattacharya, S., Tyagi, K., Srivastava, V.M., Murthy, P.K., 2003. 7-O-[4-methyl piperazine-1-(2-acetyl)]-2H-1-benzopyran-2-one: a novel antifilarial lead compound. Acta Trop. 87, 215–224.
Tripathi, R.P., Tripathi, R., Bhaduri, A.P., Singh, S.N., Chatterjee, R.K., Murthy, P.K., 2000. Antifilarial activity of some 2H-1-benzopyran-2-ones (coumarins). Acta Trop. 76, 101–106.
Twum-Danso, N.A., loa, L., 2003. Encephalopathy temporally related to ivermectin administration reported from onchocerciasis mass treatment programs from 1989 to 2001: implication for the future. Filaria J. 2, S7.