South African Journal of Botany 164 (2024) 288–296

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Antimycobacterial, hepatoprotective and cytotoxicity effects of selected plant species from the Menispermaceae family

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ARTICLE INFO

Article History:
Received 30 July 2023
Revised 27 November 2023
Accepted 28 November 2023
Available online 7 December 2023

Edited by Dr S.C. Pendota

Keywords:
Antimycobacterial
Cytotoxicity
Hepatoprotective
Menispermaceae
MDR-*Mycobacterium tuberculosis*
Cissampelos

ABSTRACT

Tuberculosis, caused by *Mycobacterium tuberculosis*, is a worldwide disease affecting millions of people. The rise of resistant strains, coupled with toxicity of current chemotherapy, requires effective alternatives against mycobacterial infections. Some plants from the Menispermaceae family have been used to treat tuberculosis and cough related symptoms. In this study, acetone, methanol:water (4:1), dichloromethane:methanol (1:1) and hot water extracts of different plant parts of *Cissampelos owariensis, Cissampelos mucronata* and *Tinospora fragosa* were tested against *M. aurum, M. bovis, M. fortuitum, M. smegmatis and M. tuberculosis* using a two-fold serial microdilution assay. Cytotoxicity of the active extracts was determined against Vero and HepG2 cells. The hepatoprotective effect of the active extracts was evaluated using rifampicin and acetaminophen as toxic drugs against HepG2 cells. The hot water leaf extracts were most active with promising minimum inhibitory concentration (MIC) values of 20 and 40 μg/mL against *M. smegmatis* and *M. fortuitum* respectively. The acetone extracts of *C. owariensis, C. mucronata* and *T. fragosa* had the lowest MIC values (0.03 – 1.67 mg/mL). The root extract of *C. owariensis* was not toxic to Vero cells while the leaf extract was more toxic. The acetone extract of *C. mucronata* leaves was toxic to Vero cells but the other extracts had low toxicity. The active leaf and root extracts had protective effects on rifampicin-induced toxicity on HepG2 cells. The root extract also had a protective effect on acetaminophen-induced toxicity on HepG2 cells but the leaf extract had no protective effect. The hot water extracts of *C. owariensis, C. mucronata* and *T. fragosa* had a more protective effect on the toxin-induced cells than the acetone extracts. These results support further investigation on the bioactive compounds in these plant extracts.

1. Introduction

Tuberculosis (TB) is the most common cause of morbidity and mortality, especially when co-infection with other disease conditions occurs (Gandhi et al., 2006). The disease is one of the top ten causes of death globally from a single infectious agent (WHO, 2019). Tuberculosis remains a serious health concern despite the available therapy i.e., directly observed treatment short course (DOTS) therapy and availability of dedicated care centres since the 1950s (Madikizela et al., 2013; Priyadarshini and Veeramani, 2020). The statistics of TB incidence and mortality rates increased recently during the Covid-19 pandemic, with close to 10.6 million active cases, and 1.4 million and 187 000 deaths among HIV (human immune deficiency virus) negative and positive people respectively (WHO, 2022). Although there is a certain level of improvement in TB/HIV co-infection, mortality among TB/HIV patients is attributed to factors such as extra-pulmonary TB and pulmonary TB co-infection, and baseline cluster of differentiation for T-lymphocyte counts <200 cells/μL at the time of HIV diagnosis (Yang et al., 2023). A quarter of the world's population could be latently infected with *Mycobacterium tuberculosis,* which is reactivated in immune suppressed conditions (WHO, 2019; Priyadarshini and Veeramani, 2020). Non-compliance of patients to the stringent and lengthy tuberculosis chemotherapy, coupled with toxicity concerns and co-infection with other diseases such as HIV and diabetes mellitus, results in genetic mutations of *M. tuberculosis*. These genetic mutations give rise to resistant strains of *Mycobacterium* species (Kahaliw et al., 2017). Drug toxicity and the persistent nature of *Mycobacterium* species have led to the need to explore and develop

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https://doi.org/10.1016/j.sajb.2023.11.048

new drugs effective against mycobacteria causing pathogenesis in humans, with a shorter treatment regimen (Mariita et al., 2010). Scientific interest in natural products such as medicinal plants has risen over the years because natural products possess morphological features similar to drug-like molecules. Nature's biosynthetic pathway creates several metabolites with various biological properties which make them inhibitors or promoters of biological action (Kahaliw et al., 2017). Promising antimycobacterial activity has been reported in extracts of many higher plants such as *Euclea natalensis, Gentianopsis paludosa* and *Thymus vulgaris* (Arya, 2011; McGaw et al., 2008).

The Menispermaceae family consists of flowering plants occurring in many parts of the world. It is a medium sized family of 70 genera with 520 extant species, comprising climbing or self-supporting plants, mostly stem twiners (Wefferling et al., 2013). Most of the genera are tropical, but a few, including *Menispermum* and *Cocculus,* are found in the temperate climates in eastern North America and eastern Asia (De Wet et al., 2004). Menispermaceae species are rich in alkaloids, such as benzyl tetrahydroisoquinoline and aporphine derivatives, which are popular globally for their medicinal uses (De Wet and Van Wyk, 2008; Barbosa-Filho et al., 2000; De Wet, 2005). Various parts of the plants are widely used in African traditional medicine (Bouquet and Debray, 1974; Watt and Breyer-Brandwijk, 1962; Oliver-Bever, 1986). Some South African Menispermaceae species are currently used in traditional medicine to treat cough, tuberculosis and related symptoms (Koenen, 1996; Van Wyk and Gericke, 2000). Preliminary investigation of some species of this family revealed good antimycobacterial activity against some *Mycobacterium* strains (Akande et al., 2013; Njeru et al., 2015; Gupta et al., 2018).

Cissampelos mucronata A.Rich. is a shrub-like climber widely distributed in tropical Africa from Senegal east to Ethiopia and southern Africa. The species grows in deciduous bushland, coastal woodlands, and cultivated land (De Wet, 2005). The roots and leaves are used to treat coughs (Van Wyk and Gericke, 2000; De Wet and Van Wyk, 2008), tuberculosis and other respiratory diseases (Aska and Kubmarawa, 2016). The leaves are used to treat scrofula associated with *M. tuberculosis* infection of the lymph nodes (Watt and Breyer-Brandwijk, 1962). The antimycobacterial effect of *C. mucronata* extracts and isolates was previously reported (*M. tuberculosis* BCG strain: 5 mg/mL; *M. smegmatis, M. fortuitum, M. aurum, M. bovis, M. tuberculosis*: 0.02 - 2.5 mg/mL; *M. smegmatis, M. bovis* BCG: 3.13 – 6.25 mg/mL) (Aska et al., 2019; Akande et al., 2022).

Cissampelos owariensis P.Beauv. ex DC. is widespread throughout tropical Africa from East Africa to West and some southern African countries. This species is a liana and grows primarily in seasonally dry tropical land or forest regions (POWO, 2023). *C. owariensis* is used locally to treat cough (Oliver-Bever, 1986). Previous reports revealed the plant had promising antimycobacterial effect (*M. tuberculosis* isolates: MIC = 3 mg/mL; *M. smegmatis, M. bovis* BCG: 0.39 - 3.13 mg/mL) (Akande et al., 2013; Ibekwe et al., 2022).

Tinospora fragosa (I.Verd.) I.Verd. & Troupin is found in West and southern Africa. Its spread in South Africa is constrained to dry subtropical regions of Northern Mpumalanga and Limpopo province. It grows in hot, dry places especially on rocky sites and near dry riverbeds. It is a twiner that grows 10 m or higher. This species can reestablish itself when its growth is interrupted by forming an aerial root 10 m or higher above the ground and re-rooting itself (Alicia and Gillian, 2017; De Wet et al., 2016). *T. fragosa* is used to prevent cough and anthrax in ethnomedicine (De Wet and Van Wyk, 2008; Shai et al., 2010). Previous reports revealed cytotoxic and antidiabetic effects (De Wet et al., 2009; Shai et al., 2011). In this study, the preliminary evaluation of the antimycobacterial, cytotoxic and hepatoprotective effects of *Cissampelos owariensis* extracts led to a more detailed investigation of the extracts of *C. owariensis* plant and aerial parts of *C. mucronata* and *T. fragosa*. This study hereby reports the antimycobacterial, cytotoxic and hepatoprotective effects of *Cissampelos owariensis, C. mucronata* and *Tinospora fragosa* of the Menispermaceae family in view of the ethnomedicinal use of species of this family for cough and tuberculosis infection.

2. Materials and methods

2.1. Plant collection and authentication

Cissampelos owariensis plant material was collected from Damaturu, Yobe state and *C. mucronata* from Idu, Abuja, Nigeria. Both species were collected in March 2019. *Cissampelos owariensis* was authenticated and the voucher specimen (NIPRD/H/6569) was deposited at the National Institute of Pharmaceutical Research and Development Herbarium Unit, Abuja, Nigeria. *Tinospora fragosa* was collected at the Hatfield campus of the University of Pretoria, South Africa. *Cissampelos mucronata* and *Tinospora fragosa* were identified and authenticated at the H.G.W.J. Schweickerdt Herbarium, University of Pretoria, South Africa. The voucher specimen numbers were PRU 0,125,290 and PRU 125,489 respectively.

2.2. Extraction of plant material

Cissampelos owariensis (leaves and roots) were air dried and ground into powder. The powdered materials were separately extracted with acetone (100 %), methanol:water (4:1) and hot water (1:10 W/V) (Lall et al., 2016). In a second extraction procedure, the whole plant of *Cissampelos owariensis*, as well as the aerial parts of *Tinospora fragosa*, leaves and stem of *Cissampelos mucronata* were also air dried and ground into powder. These ground plant materials were separately extracted with the above-mentioned solvents and also dichloromethane:methanol (1:1) (1:10 W/V) (Lall et al., 2016). The extracts were filtered through Whatman No.1 filter paper and concentrated under a stream of cold air. The water extracts were freeze dried. The extracts were weighed and stored at 2 – 8 °C until use.

2.3. Preparation of extracts

Acetone, sterile water and 10 % dimethylsulfoxide (DMSO) were used to dissolve extracts where appropriate. A stock solution of 10 mg/mL for each extract was prepared for antimycobacterial assays. The extracts were streaked on Mueller Hinton agar and incubated at 37 °C for 18 - 24 h to check for contamination before use.

2.4. Minimum inhibitory concentration of C. owariensis, C. mucronata and T. fragosa extracts

The minimum inhibitory concentration (MIC) of the extracts was determined against fast growing strains according to the method of Tran et al. (2017) with slight modifications. The test inoculum (*M. smegmatis, M. fortuitum, M. aurum*) was grown in Middlebrook 7H9 broth supplemented with Tween 80 and 10 % OADC supplement (Sigma Aldrich, Johannesburg, South Africa). The cultures were grown at 37 °C for 24 h (*M. smegmatis*), 48 h (*M. fortuitum*) and 5 days (*M. aurum*). Single colony suspensions of the cultures were prepared. The cultures were diluted to OD_{600} = 0.004 (1.12×10^6) in 7H9 medium. A two-fold serial dilution was performed using equal volumes (100 μL) of extracts and 7H9 medium. Streptomycin and rifampicin were used as positive controls at a concentration range of 25 – 0.20 μg/mL. DMSO (10 %), bacterial culture and OADC supplemented medium served as negative controls (other solvents used for extracts preparation were included as a negative control in the applicable plate). One hundred μL of the mycobacterial culture were added to all the wells. The plates were sealed in a plastic bag and incubated under the same conditions as stated above for each strain.

Mycobacterium tuberculosis (TB 899A) and *M. bovis* (TB 9350C) field strains (obtained from ARC—OVR Tuberculosis Laboratory,

Pretoria, South Africa) and reference *M. tuberculosis* (ATCC 25,177) were grown on Lowenstein-Jensen (LJ) slants supplemented with glycerol (*M. tuberculosis* growth supplement), or pyruvate in the case of *M. bovis*, for 3 - 4 weeks. The colonies were sub-cultured in Middlebrook 7H9 medium for 14 - 15 days. The test inoculum was prepared in sterile water and adjusted to McFarland No. 1 standard solution (3×10^8 CFU/mL). The cultures were diluted to a final density of 5×10^5 CFU/mL with freshly prepared supplemented Middlebrook 7H9 medium. Sterile distilled water (200 μL) was added to the outer wells to reduce evaporation. Two-fold serial dilutions were performed using 100 μL of extracts and 100 μL 7H9 medium. The positive and negative controls (as stated above) were included. One hundred μL of the mycobacterial culture were added to all the wells. The plates were sealed in a plastic bag and incubated for 7 days (*M. bovis, M. tuberculosis* strains) at 37 °C. MIC values were determined by adding 40 μL of 0.2 mg/mL of freshly prepared thiazolyl blue tetrazolium bromide (MTT, Inqaba Biotec, South Africa) as growth indicator (McGaw et al., 2008; Bhunu et al., 2017). The colour reaction occurred after incubation within 60–90 min for fast growing mycobacteria and within 6 h for pathogenic strains. The results were read as soon as a purple color became visible in the untreated control wells. MIC values were recorded as the concentrations with a decrease in colour formation corresponding to inhibition of mycobacterial growth. The assays were run in triplicate and the experiments were repeated twice.

2.5. Thin layer chromatography analysis and bioautography of extracts

Thin layer chromatography and bioautography of the extracts with antimycobacterial activity in the MIC assay was carried out according to the method of Begue and Kline (1972). The extracts included the acetone extracts of *Cissampelos mucronata, C. owariensis* and *Tinospora fragosa*. Hexane:acetone (7:3) and toluene:ethyl acetate:acetic acid (7:2:1) were used as the mobile phases according to the method of Gunasekaran et al. (2016) with modified ratios. The presence of different phytochemicals in the extracts was highlighted by spraying eluted TLC plates with vanillin-sulphuric acid spray reagent and heating the plates at 110 °C for optimal colour development. Dragendorff spray reagent was used to detect alkaloids in the extracts. Bioautography using *M. smegmatis* was performed to determine the active compounds in the extracts by preparing duplicate chromatograms as described in the above-mentioned assay. The duplicate plate was left in the dark and uncovered under a stream of cold air for 24 h to evaporate the solvent on the plate before being sprayed with actively growing suspension of *M. smegmatis* (cultured for 18 – 24 h at 37 °C). The moist plates were incubated at 37 °C in a closed sterile plastic container for 24 h to aid bacterial growth on the plates. The plates were then sprayed with 2 mg/mL of p-iodonitrotetrazolium violet (INT) (Sigma, Johannesburg, South Africa) and incubated for 1 h. Clear zones against the purple-red background were indicative of the active compounds inhibiting the bacterial growth.

2.6. Cytotoxicity assay and selectivity index

The cytotoxicity of the crude plant extracts was determined against Vero African green monkey kidney cells (ATCC CCL-81) using the MTT assay (Mosmann, 1983) with slight modifications (McGaw et al., 2007). The cytotoxic effects of the active extracts were also determined against HepG2 liver cells (ATCC HB-8065). Cytotoxic effect of the active extracts on the HepG2 cells was carried out to determine the safe concentration for hepatoprotective effects of the extracts. The Vero cells were maintained in minimal essential medium (MEM, PAN Biotech, Biocom Africa, South Africa) supplemented with 5 % foetal bovine serum (Biocom Africa) and 0.1 % gentamicin (Virbac) while HepG2 cells were maintained in Dulbecco's Modified Eagle media (DMEM, Pan Biotech, Separation Scientific, South Africa) supplemented with 10 % foetal bovine serum and 1 % of 100 IU/mL penicillin and 100 μg/L streptomycin (Pan Biotech, Celtic Diagnostics, South Africa). The cell suspensions were prepared from confluent monolayer cultures and plated at a density of 1×10^4 for the Vero cells and 2×10^4 for the HepG2 cells in 96-well microtitre plates. The plates were incubated for 24 h at 37 °C in a 5 % CO_2 incubator prior to exposure. The crude plant extracts were prepared in acetone (for the acetone extracts), sterile water (hot water extracts) and 50 % DMSO (methanol:water and dichloromethane:methanol extracts) at 100 mg/mL and appropriate dilutions were prepared with MEM and DMEM for Vero and HepG-2 cells respectively and added to the cells. The cells were exposed to various concentrations (0.0075 – 1 mg/mL) of plant extract for 48 h. Doxorubicin (Pfizer Laboratories, South Africa) was used as a positive control. After 48 h incubation, the wells were rinsed with 200 μL of phosphate buffered saline (PBS, Sigma) and 100 μL of fresh medium was dispensed into the wells. Thirty microliters of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 5 mg/mL, Inqaba Biotec, South Africa) dissolved in PBS was added to each well and incubated for 4 h at 37 °C. The medium was removed and MTT formazan crystals were dissolved with 50 μL DMSO. Absorbance was measured using a microplate reader (BioTek Synergy) at a wavelength of 570 nm. Each concentration was tested in triplicate and the assay was repeated three times. The half-maximal lethal concentration (LC_{50}) of the extracts was calculated. The selectivity index (SI) was determined by dividing cytotoxicity (LC_{50}) by the MIC values (LC_{50}/MIC).

2.7. Hepatoprotective assay

The hepatoprotective effect of the active extracts was determined using HepG2 liver cells treated with rifampicin and acetaminophen separately as toxic drugs according to González et al. (2017). The liver cells (HepG2) (2×10^4) were seeded in 100 μL media (DMEM) in a 96-well plate and incubated for 24 h at 37 °C and 5 % CO_2 environment. The toxicants (acetaminophen 40 mM; rifampicin 1 mg/mL), different concentrations of the extracts and positive control (silymarin; 0.0075 mg/mL) were prepared and added to the cells. The control cells were treated with the toxicants alone. The plates were incubated for 24 h at 37 °C and 5 % CO_2. The medium was removed after incubation and the cells were washed with PBS (200 μL). Then 100 μL of media was added and MTT (5 mg/mL in PBS; 30 μL) was also added to the cells and incubated for 2 h at 37 °C and 5 % CO_2 incubator. The medium was removed, 50 μL DMSO was added to dissolve the MTT formazan crystals and the absorbance read at 570 nm. Percentage cell viability was calculated as follows:

$$\text{Average of absorbance of treated cells} / \text{average of absorbance of control cells} \times 100$$

2.8. Statistical analysis

Data was presented as mean ± standard deviation (MIC and cytotoxicity assay). Hepatoprotective assay results were expressed as mean ± standard deviations and statistically significant values were compared using one way ANOVA analysis of variance with an interactive statistical program (Sigmastat, SPSS version 26, USA). Standard error of mean was used to calculate the error bars.

3. Results

3.1. Antimycobacterial effect of extracts

Cissampelos owariensis leaf extract had significant activity compared to the root extract (Table 1). The hot water leaf extract had minimum inhibitory concentration (MIC) values of 0.04, 0.02 and

Table 1
Minimum inhibitory concentration of extracts against fast growing and pathogenic Mycobacterium strains (mg/mL).

Plant part	Solvent	*M. smegmatis* (ATCC 1441)	*M. fortuitum* (ATCC 6841)	*M. aurum* (NCTC 10,437)	*M. bovis* (TB 9350C)	*M. tuberculosis* (TB8993A)	*M. tuberculosis* (ATCC 25,177)
Cissampelos mucronata (leaves)	Acetone	**0.03±0.01**	**0.06±0.03**	**0.06±0.03**	0.16±0	0.13±0.05	0.13±0.05
	Methanol:water (4:1)	0.31±0	0.63±0	**0.04±0**	0.31±0	1.88±0.88	0.63±0
	Hot water	0.63±0	0.63±0	**0.015±0.007**	**0.04±0**	**0.015±0.007**	**0.01±0**
	Dichloromethane:methanol (1:1)	0.63±0	0.63±0	**0.04±0**	0.16±0	0.63±0	0.26±0.09
Cissampelos mucronata (stem)	Acetone	0.16±0	**0.06±0.03**	**0.08±0**	0.31±0	0.16±0	0.13±0.05
	Methanol:water (4:1)	0.63±0	2.5 ± 0	0.63±0	1.04±0.36	2.5 ± 0	0.42±0.19
	Hot water	0.31±0	0.47±0.19	0.47±0.23	0.94±0.44	2.5 ± 0	2.5 ± 0
	Dichloromethane:methanol (1:1)	0.63±0	2.5 ± 0	**0.06±0.03**	2.5 ± 0	0.94±0.44	2.5 ± 0
Cissampelos owariensis (whole plant)	Acetone	0.16±0	0.24±0.09	**0.03±0.01**	0.94±0.44	0.13±0.05	0.31±0
	Methanol:water (4:1)	0.63±0	1.88±0.88	0.31±0	0.31±0	1.25±0	0.94±0.44
Cissampelos owariensis (leaves)	Hot water	0.63±0	0.47±0.23	0.31±0	0.94±0.44	0.47±0.23	0.52±0.19
Cissampelos owariensis (roots)	Dichloromethane:methanol (1:1)	0.31±0	0.63±0	0.31±0	1.88±0.88	0.94±0.44	1.88±0.88
	Acetone	>2.5	>2.5	2.5	-	-	-
	Methanol:water (4:1)	1.25	0.16	1.25	-	-	-
	Hot water	**0.02**	**0.04**	0.625	-	-	-
	Acetone	2.5	1.25	1.25	-	-	-
	Methanol:water (4:1)	2.5	1.25	0.625	-	-	-
	Hot water	>2.5	2.5	2.5	-	-	-
Tinospora fragosa (aerial parts)	Acetone	0.12±0.05	**0.08±0**	0.16±0	0.63±0	0.84±0.36	1.67±0.72
	Methanol:water (4:1)	1.25±0	0.79±0.31	0.31±0	1.88±0.88	0.31±0	2.08±0.72
	Hot water	0.31±0	0.31±0	0.16±0	0.63±0	0.63±0	1.25±0
	Dichloromethane:methanol (1:1)	0.63±0	0.31±0	**0.08±0**	1.88±0.88	1.88±0.88	0.63±0
Standards	Rifampicin	0.0047±0.23	**0.0006±0.03**	**0.0004±0**	**0.0002±0**	0.0063±0	**0.0003±0.01**
	Streptomycin	**0.0002±0**	0.0047±0.23	**0.0002±0**	**0.0003±0.01**	**0.0002±0**	**0.0003±0.01**

0.625 mg/mL against *M. fortuitum, M. smegmatis* and *M. aurum* respectively. Extracts with MIC less than 0.1 mg/mL are considered significantly active, MIC >0.1 to 0.625 mg/mL, is moderately active, and weakly active if MIC>0.625 mg/mL (Kuete, 2010). The aqueous methanol leaf extract had MIC of 0.16 mg/mL against *M. fortuitum* and the root extract had a moderate activity of 0.625 mg/mL against *M. aurum*. The MIC results of *C. mucronata, C. owariensis* and *T. fragosa* extracts are presented in Table 1. The hot water and acetone extracts of *C. mucronata* had the best activity against the strains (Table 1). *Cissampelos mucronata* leaf extract was more active than the stem extract. The acetone leaf extract of *C. mucronata* was more active (MIC = 0.03–0.16 mg/mL) than the hot water extract (MIC = 0.01–0.630 mg/mL). The same pattern was observed with the stem extracts (MIC: acetone; 0.06–0.31 mg/mL; hot water; 0.31–2.5 mg/mL). Promisingly, the lowest MIC values of the hot water extracts were close to the MIC values of the positive controls (rifampicin and streptomycin; 0.2–6.3 μg/mL).

3.2. Thin layer chromatography and bioautography

The spray reagents revealed compounds of different polarities on the TLC plates. More than 10 compounds were separated on the plates with the solvent systems [hexane:acetone (7:3) and toluene:ethyl acetate:acetic acid (7:2:1)]. Both solvent systems gave good separation. Some compounds in the extracts had common or close retention factors [Rf; 0.55 (hexane:acetone 7:3); 0.56 (toluene:ethyl acetate:acetic acid 7:2:1)]. The bioautography of the plate eluted with toluene:ethyl acetate:acetic acid (7:2:1) showed active bands in extracts of *C. mucronata* leaves and stem and *C. owariensis* whole plant. Hexane:acetone (7:3) showed active bands with *C. mucronata* leaves and stem (Figures S1 and S2; supplementary data). Some compounds were visible as orange bands with Dragendorff reagent (Kokotkiewicz et al., 2017) (Figures S1 and S2; supplementary data). The Rf of one of the antibacterially active bands (0.71: *C. owariensis*, toluene:ethyl acetate:acetic acid (7:2:1)) corresponded to the Rf (0.75) of a band on the TLC plate sprayed with Dragendorff reagent.

3.3. Cytotoxicity of extracts and selectivity index

Cissampelos owariensis hot water leaf extract was toxic to Vero cells with an LC_{50} of 0.01 mg/mL comparable to that of doxorubicin, the positive control (LC_{50} = 0.01 mg/mL) (Table 2). A plant extract is thought to be cytotoxic when the LC_{50} is 0.020 mg/mL and below (Kuete et al., 2011). The same extract was relatively less toxic to the liver cells while the root extract was less toxic to the liver and Vero cell lines. The cytotoxic effect of the extracts with HepG2 cells was conducted to determine the optimal concentration for the hepatoprotective effect of the extracts. The cytotoxicity of *C. owariensis, T. fragosa* and *C. mucronata* extracts is presented in Table 2. The acetone extract of *C. mucronata* leaves was relatively toxic (LC_{50} = 0.02 mg/mL) to Vero cells. The acetone extracts of *C. mucronata* stem, *C. owariensis* and *T. fragosa* had low toxicity to Vero cells (LC_{50} = 0.03, 0.06, 0.04 mg/mL respectively). Hot water, methanol:water and dichloromethane:methanol extracts also exhibited low toxicity to the cells (LC_{50} = 0.05 – 1 mg/mL). *Cissampelos mucronata* leaf extract (hot water extract) had the best selectivity index (SI = 30) with regards to the *Mycobacterium* strains. The SI value is a reflection of the selective toxicity of the extract to the bacteria, compared to toxicity to normal mammalian cells. This means that the higher the SI, the more antibacterial the extract, in the absence of general toxicity to mammalian cells. The methanol:water and dichloromethane:methanol extracts had SI values of 6.5 and 2.5 respectively with *M. aurum. Tinospora fragosa* also had a high SI followed by *C. owariensis*.

3.4. Hepatoprotective effect of the extracts

Cissampelos owariensis root and leaf extracts had protective effects to the liver cells (HepG2) after exposure to the two toxicants when compared to silymarin (positive control) and the control cells (Figs. 1 and 2). There was a significant reduction in cell viability with both toxins when compared to the normal non-stimulated cells (RIF: $p < 0.001$; ACET: $p = 0.003$; Sigmastat, SPSS version 26, USA). Silymarin significantly increased the viability of the toxin-induced cells in comparison to the control cells with $p<0.001$. The root and leaf extracts increased the viability of RIF-induced cells significantly at

Table 2
Cytotoxicity and selectivity index values of extracts.

Plant	Solvent/Extract	LC_{50} (mg/mL)	Selectivity index (Vero cells)					
			Ms	*Mf*	*Ma*	*Mb* (TB9650C)	*Mtb* (TB8993A)	*Mtb* (ATCC25177)
C. mucronata (leaves)	Acetone	0.02± 0.01	0.67	0.33	0.33	0.125	0.15	0.15
C. mucronata (stem)		0.03±0	0.19	0.5	0.38	0.1	0.19	0.23
C. owariensis (plant)		0.06±0.02	0.38	0.25	**2**	0.06	0.46	0.19
T. fragosa (aerial part)		0.04±0.01	0.33	0.5	0.25	0.06	0.05	0.02
C. mucronata (leaves)	Hot water	0.30±0.13	0.48	0.48	**20**	**7.5**	**20**	**30**
C. mucronata (stem)		0.12±0.006	0.39	0.26	0.26	0.13	0.05	0.05
C. owariensis (plant)		0.72±0.09	1.14	1.53	**3.23**	**2.32**	1.53	1.39
T. fragosa (aerial part)		>1000	**3.23**	**3.23**	**6.25**	1.59	1.59	0.8
C. owariensis (leaves)		0.01±0.01	0.72	0.36	0.02	-	-	-
C. mucronata (leaves)	Methanol:water (4:1)	0.26±0.11	0.84	0.41	**6.5**	0.84	0.14	0.41
C. mucronata (stem)		0.34±0.05	0.54	0.14	0.54	0.33	0.14	0.81
C. owariensis (plant)		0.16±0.03	0.25	0.09	0.52	0.52	0.13	0.17
T. fragosa (aerial part)		0.19±0.04	0.15	0.24	0.61	0.1	0.61	0.09
C. owariensis (roots)		0.16±0.05	0.06	0.13	0.25	-	-	-
C. mucronata (leaves)	Dichloromethane:	0.1 ± 0.03 0.05±0.01						
C. mucronata (stem)	methanol (1:1)	0.05±0.02						
C. owariensis (plant)		0.49±0.19						
T. fragosa (aerial part)								
Doxorubicin	Standard	0.0102±0.0025						

0.01, 0.05 and 0.075 mg/mL (root: $p < 0.001$; leaves: $p < 0.001$, $p = 0.008$ and $p < 0.001$ respectively). The root extract increased the viability of ACET-induced cells at all concentrations tested (0.01, 0.05 and 0.075 mg/mL; $p<0.001$, $p = 0.001$ and $p = 0.006$ respectively). However, treatment with the leaf extract resulted in no statistical significance, i.e., treatment with the leaf extract did not attenuate the ACET induced toxicity in the study (Table 3). The active extracts (acetone and hot water extracts of the Menispermaceae species from the antimycobacterial assay) had hepatoprotective effects on HepG2 cells compared to the positive control (silymarin), control cells and the normal cells ($p < 0.05$; a p-value of ≤0.05 was considered statistically significant) (Figs. 3 and 4, Table 4). There was a reduction in cell viability observed with the control cells, and treatment with the extracts led to an increase in cell viability (Table 4). There was a significant increase in cell viability at the lowest concentration (0.025 mg/mL) ($p < 0.05$, for extracts treated with toxin-induced cells; Sigmastat, SPSS version 26, USA). The hepatoprotective effect was concentration-dependent with most of the extracts (Table 4). A drastic reduction in cell viability was observed at the highest concentration (0.1 mg/mL) with the acetone extracts ($p < 0.05$) (Table 4). The hot water extract displayed a more protective effect than the acetone extract (Figs. 3 and 4; Table 4).

A(CM, CML, CMS, COW, TFA) – Acetone extracts (*C. mucronata* aerial part, *Cissampelos mucronata* leaves, *Cissampelos mucronata* stem, *Cissampelos owariensis* plant, *Tinospora fragosa* aerial part); H (CMS, COW, CML, TFA) – Hot water extracts (*Cissampelos mucronata* stem, *Cissampelos owariensis* plant, *Cissampelos mucronata* leaves, *Tinospora fragosa* aerial parts)

4. Discussion

The antimycobacterial assay revealed similar patterns of activity for *C. owariensis* leaf and root extracts as well as for the aerial parts of *C. mucronata,* attributed to the presence of similar phytochemicals in the plant extracts (Ndukwe et al., 2007). A similar pattern of activity was also observed for *C. mucronata* leaf and stem extracts. Acetone extracts a wide range of polar and non-polar compounds from plants, while water extracts are comprised of largely polar compounds (Borges et al., 2020). The bioautography assay in this study revealed non-polar active zones, implying that the bioactive compounds are mainly relatively non-polar.

Cissampelos mucronata had a wide range of activity with MIC values ranging from 0.01 to 1.88 mg/mL for the leaves and 0.06 – 2.5 mg/mL for stem extracts against *Mycobacterium* spp. A study of

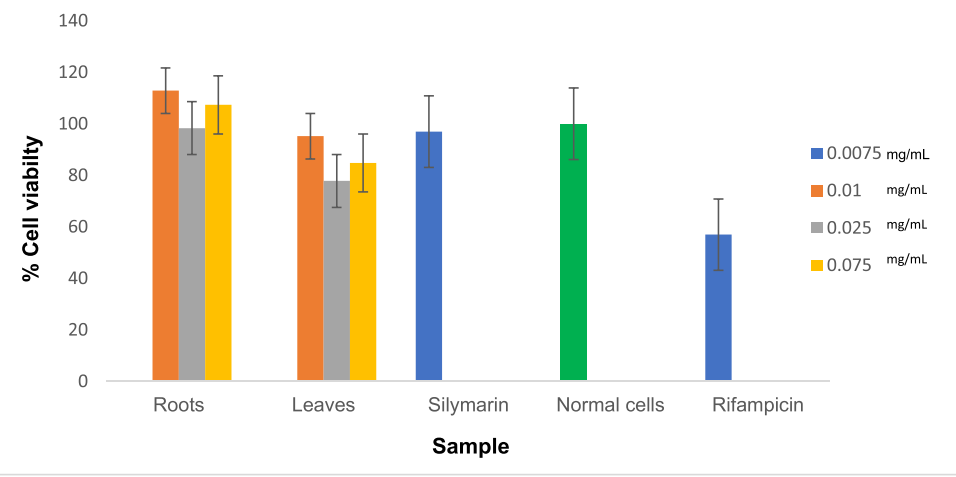

Fig. 1. Hepatoprotective effect of C. owariensis extracts using HepG2 cells with rifampicin as the toxin.

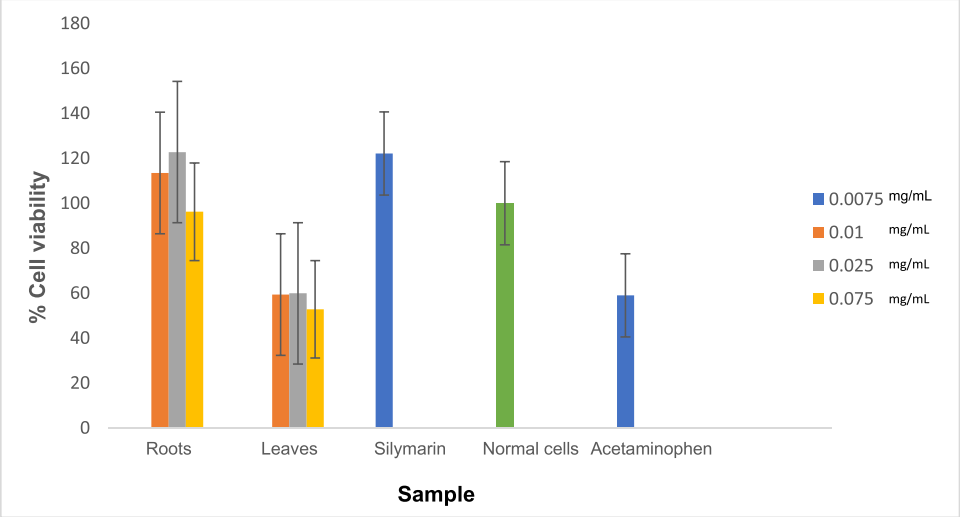

Fig. 2. Hepatoprotective effect of *C. owariensis* extracts using HepG2 cells with acetaminophen as the toxin.

the ethanolic and dichloromethane extracts of the aerial part and root of this plant by Nondo et al. (2011) revealed moderate antibacterial activity (MIC: 0.391 - 12.5 mg/mL; 0.195 - 25 mg/mL respectively) against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera, Bacillus anthracis* and *Streptococcus faecalis*. This indicates that *C. mucronata* has broad spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. Aska et al. (2019) reported that the aqueous methanol root extract of *C. mucronata* had poor activity against *M. tuberculosis* BCG (MIC = 5 mg/mL). Apart from potential variabilities in bioactive phytochemicals in the plant parts observed in similar studies above, differences in the geographical area or seasonal changes might also be responsible for the differences in the biological activity observed in these studies (Oosthuizen et al., 2019). *C. mucronata* is used in ethnomedicine for the treatment of tuberculosis, cough and fever (Van Wyk and Gericke, 2000; De Wet and Van Wyk, 2008; Aska and Kubmarawa, 2016). The findings of this study support the ethnomedicinal use of the plant for tuberculosis related symptoms.

Cissampelos owariensis generally exhibited moderate activity (MIC = 0.02 – 1.88 mg/mL). A preliminary study conducted by Akande et al. (2013) revealed that the methanolic root extract of *Cissampelos owariensis* was active at 3 mg/mL against some *M. tuberculosis* isolates evaluated by drug susceptibility test using an indirect proportion method. Akande et al. (2013) also reported moderate to poor antimicrobial activity against *Staphylococcus aureus, S. typhi, E. coli, Pseudomonas aeruginosa* (MIC = 0.125 - 0.250 mg/mL; 12.5 mg/mL) with the compounds and methanolic root extract respectively. The previous study on *C. owariensis* corroborates the antibacterial effect observed in this study. In addition to its diverse use in traditional medicine (Earnest et al., 2015), *C. owariensis* is also used for tuberculosis-related symptoms i.e., persistent cough and fever (Oliver-Bever, 1986). *C. mucronata, C. owariensis* and *C. pareira* have often been confused as they have similar uses, and it is often difficult to differentiate these species on the basis of their ethnomedicinal uses (Muzila et al., n.d.). Similar phytochemical constituents in the related species could contribute to the similarity in their ethnomedicinal use and the antimycobacterial effect observed in this study.

Tinospora fragosa had moderate activity (MIC = 0.08 – 2.08 mg/mL). *T. fragosa* is used traditionally to treat cough and anthrax sores, and is given to cattle as fodder to prevent anthrax (De Wet and Van Wyk, 2008). Biological activity reported for *T. fragosa* includes alpha-glucosidase inhibition and cytotoxic effects, but there has been no report of its antimicrobial effect (De Wet et al., 2009; Shai et al., 2010). The ethnomedicinal use of the plant for bacterial infection such as anthrax is an indication that *T. fragosa* might have possible antimicrobial effects, which is supported by the antimycobacterial

Table 3
Hepatoprotective and cytotoxic effects of *Cissampelos owariensis* root and leaf extracts with HepG2 cells.

% cell viability of HepG2 cells					
Conc. (mg/mL)	Roots	Leaves	Silymarin	Rifampicin	Normal cells
0.0075	-	-	96.965	56.95202	100
0.01	112.85	95.18	-	-	-
0.025	98.315	77.82	-	-	-
0.075	107.335	84.805	-	-	-

% cell viability of HepG2 cells					
Conc. (mg/mL)	Roots	Leaves	Silymarin	Acetaminophen	Normal cells
0.0075	-	-	122.105	58.98313	100
0.01	113.455	59.325	-	-	-
0.025	122.73	59.875	-	-	-
0.075	96.185	52.795	-	-	-

Cytotoxicity			
LC_{50} (mg/mL)	0.19±0.05	0.13±0.05	0.0020±0.0011 (Doxorubicin)

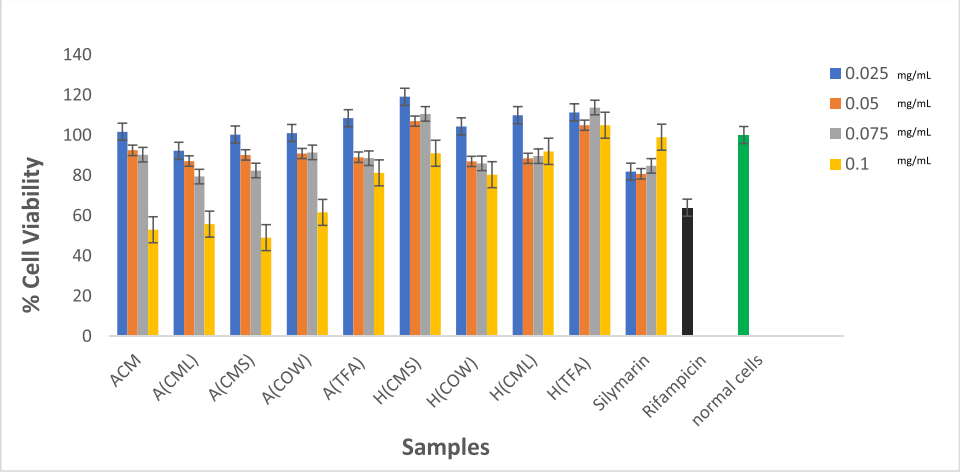

Fig. 3. Hepatoprotective effect of active extracts with HepG2 cells using rifampicin as the toxin.

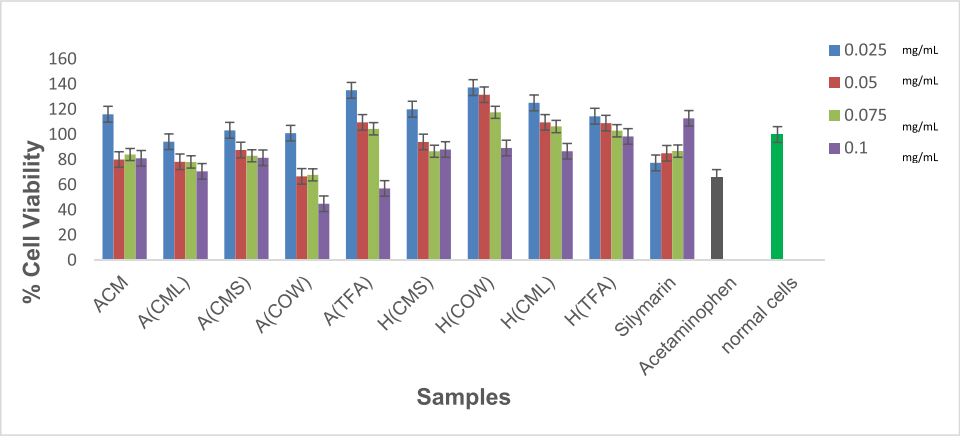

Fig. 4. Hepatoprotective effects of active extracts with HepG2 cells using acetaminophen as the toxin.

activity observed in this study. *B. anthracis,* the causative agent of anthrax, is susceptible to some tuberculosis drugs (Bryskier, 2002). *B. anthracis* (Beesley et al., 2010) and *M. tuberculosis* are both Gram-positive bacteria, although *M. tuberculosis* has a more complex cell wall (Kalscheuer et al., 2019). This is another link between the ethnomedicinal use and antimycobacterial effect observed in this study. To the best of our knowledge, this appears to be the first study on the antimycobacterial effect of *Tinospora fragosa.*

Based on the MIC results and the work of Rahman et al. (2014) and Brown-Elliott and Philley (2017) on the classification of *Mycobacterium* strains, *C. mucronata* could be useful for tuberculosis chemotherapy while *C. owariensis* and *T. fragosa* might be more effective against opportunistic infections. These findings reveal a correlation between the ethnomedicinal use and scientific evaluation of *Cissampelos* species and support the ethnomedicinal use of *C. mucronata* and *C. owariensis* for cough and tuberculosis related symptoms.

The TLC and bioautographic assay reveal similar bioactive compounds between the *Cissampelos* extracts. This implies some compounds are common to the extracts and indicates their chemotaxonomic relationship. Some compounds visible with Dragendorff reagent had similar Rf values to some of the active bands. This might be indicative of alkaloids and supports the hypothesis that the presence of alkaloids in the extracts could be responsible for the antimycobacterial activity.

Selectivity index is the ratio of cytotoxicity (LC_{50}) to activity (MIC). Selectivity index indicates the relative safety of the extract, and the higher SI index, the safer the extract (Bagla et al., 2014, Oosthuizen et al., 2019). The SI of the leaf and root extracts of *C. owariensis* is less than 1 (Table 2). Plant extracts with SI less than 1 imply the extracts are more toxic to the mammalian cells than the pathogens. This implies the extracts might exhibit general toxicity to normal cells. The extracts or plant can be considered for use in synergy with the current antituberculosis drugs which could reduce their cytotoxic effects, or potentially as a source of bioactive molecules. Menispermaceae species are rich in alkaloids (particularly bisbenzylisoquinoline alkaloids). Bisbenzylisoquinoline alkaloids are known for their numerous biological activities including anticancer, antifungal and antibacterial, anti-plasmodial, antiparasitic, cytotoxic, acetylcholinesterase inhibition, antiplatelet aggregation, vasodilator effects and histamine release inhibition (Sureram et al., 2012, Weber and Opatz, 2019). These alkaloids might be responsible for the antimycobacterial effect and cellular toxicity observed in this study (Cavalcanti da Silva et al., 2012, Weber and Opatz, 2019, Bhagya et al., 2019, Rukachaisirikul et al., 2021). However, cellular toxicity is not a true measure of the toxicity of the extracts, and *in vivo* animal toxicity studies are still needed (Famuyide et al., 2019). As stated above, high SI implies the extract is safe *in vitro* and has the potential to be developed into a safe herbal product (Famuyide et al., 2019). Therefore, crude extracts with higher SI values might be safer *in vivo* because the extract is more toxic to the pathogens than the mammalian cells (Aro et al., 2015). The high SI observed with *Cissampelos mucronata* extracts (Table 2) against most of the strains implies the plant is far more effective against the mycobacterial species than the mammalian cells and it is a good candidate for further studies. Based on this study, *T.*

Table 4
Hepatoprotective and cytotoxic effect of active extracts using HepG2 cellsA(CM, CML, CMS, COW, TFA) – Acetone extracts (*Cissampelos mucronata* aerial part, *Cissampelos mucronata* leaves, *Cissampelos mucronata* stem, *Cissampelos owariensis* plant, *Tinospora fragosa* aerial part); H(CMS, COW, CML, TFA) – Hot water extracts (*Cissampelos mucronata* stem, *Cissampelos owariensis* plant, *Cissampelos mucronata* leaves, *Tinospora fragosa* aerial part).

	% cell viability of HepG2 cells											
Conc. (mg/mL)	ACM	A(CML)	A(CMS)	A(COW)	A(TFA)	H(CMS)	H(COW)	H(CML)	H(TFA)	Silymarin	Acetaminophen	Normal cells
0.025	116.1332	94.2970	103.3472	101.0755	135.1700	120.1113	137.3965	125.1942	114.5344	77.4160	65.9449	100
0.05	80.0700	78.2702	87.7228	66.7597	109.7393	94.1129	131.6277	109.7141	109.0867	85.1449		
0.075	84.0625	78.1929	83.0591	67.9082	104.5694	86.6780	117.6379	106.4189	103.019	86.8241		
0.1	81.0582	70.6988	81.5970	44.9080	57.0660	88.1855	89.2417	86.6260	98.5614	112.7943		
	% cell viability of HepG2 cells										Rifampicin	Normal cells
0.025	101.6665	92.2409	100.2865	101.0365	108.4508	119.0732	104.3106	109.9305	111.3357	81.8631	63.9046	100
0.05	92.4761	87.0918	90.1530	90.8579	89.0092	106.9577	86.9138	88.4512	104.9629	80.7522		
0.075	90.2476	79.3945	82.3716	91.4105	88.5370	110.5218	85.9503	89.5444	113.7126	84.7423		
0.1	52.8967	55.7089	49.0152	61.5465	81.2436	90.9723	80.2983	91.9416	104.9153	99.0027		
	Cytotoxicity									**Doxorubicin**		
LC_{50} (mg/mL)	-	0.48±0.13	0.53±0.04	0.88±0.25	0.63±0.05	>1	>1	>1	>1	0.0020± 0.0011	-	-

fragosa and *C. owariensis* can also be considered for continued investigation. To the best of our knowledge, this is the first study on the *in vitro* cytotoxic effect of *C. owariensis*.

Some studies reveal that hepatotoxic effects of the first line tuberculosis chemotherapy may lead to inconsistency in chemotherapy and resistant strains among other effects in aged patients, extrapulmonary TB and TB/HIV co-infected persons (Molla et al., 2021; Okaiyeto et al., 2018). Medicinal plants that are active against mycobacterial strains would have enhanced benefit if they had hepatoprotective effects, as many current anti-tuberculosis drugs have liver-damaging properties. Human hepatoma cells are used as models for human hepatocytes for *in vitro* hepatoprotective studies (González et al., 2017). The HepG2 hepatoma cell line is widely used in liver function, metabolism and drug toxicity studies. The cells possess many biochemical and morphological features of normal liver cells, hence the need to use them for investigation of the hepatoprotective effects of medicinal plants (González et al., 2017). The extracts displayed a general protective effect on the toxin-induced cells (Table 3 and 4). The phytochemical constituents present in the leaves and acetone extract treated cells (Tables 3 and 4) could be responsible for the decrease in cellular viability. In the study by Darvin et al. (2018) using liver cells and MTT assay, tiliamosine, a bisbenzylisoquinoline alkaloid isolated from *Tiliacora racemosa* was not cytotoxic up to 100 μM, and it had GI_{25} (25 % growth inhibition) value of 264.28 μM. The ability of bisbenzylisoquinoline alkaloids in Menispermaceae species to trigger different biological activities could be responsible for the hepatoprotective effect observed in this study (Darvin et al., 2018; Weber and Opatz, 2019). This is the first record of the *in vitro* hepatoprotective effect of *Cissampelos mucronata, Tinospora fragosa* and *C. owariensis* extracts. The extracts were not cytotoxic to the liver cells (Tables 3 and 4).

5. Conclusion

The antimycobacterial effect of species of the Menispermaceae family has not been studied extensively despite the local use of many species from the family to treat cough and other symptoms of tuberculosis. *C. owariensis* and *C. mucronata* aerial parts showed significant antimycobacterial effect against selected mycobacterial strains. The extracts of three plant species were generally not cytotoxic, except for the acetone extract of *C. mucronata* and aqueous extracts of *C. owariensis*. When investigating selective activity, it was apparent that the antimycobacterial effect observed with *C. mucronata, C. owariensis* and *T. fragosa* (hot water extracts; Table 2) was not due to general toxicity. The extracts of the three plants displayed good hepatoprotective effects to liver cells in this study. This is commendable because current tuberculosis chemotherapy has toxicity concerns. An efficacious herbal drug with good safety profile and hepatoprotective effect is significant in tuberculosis chemotherapy. In particular, the potent antimycobacterial activity of the *Cissampelos mucronata* leaf extract, the safety profile of the aqueous and other extracts, hepatoprotective effect and high selectivity index suggest that the plant could be a potential source of antimycobacterial agents or treatment adjuvants. Synergistic investigation of the leaf extracts of *C. owariensis* is recommended with the current first line antituberculosis drugs to reduce their toxic effects. The *Cissampelos* species (*C. mucronata* and *C. owariensis*) currently used in ethnomedicine for cough and tuberculosis related symptoms might be relatively safe for such use (as decoctions or infusions) following further studies. *T. fragosa* also displayed a moderate antimycobacterial effect and was not cytotoxic, showing protective effects to liver cells with a high selectivity index against some strains. These preliminary results provide rationale for further studies of these Menispermaceae species.

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.

Acknowledgment

The National Research Foundation (NRF, South Africa) provided funding to support the research (Incentive Funding to LJM).

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.sajb.2023.11.048.

References

Akande, R., Famuyide, I., Makhubu, F., Nkadimeng, S., Aro, A., Kayoka-Kabongo, P., McGaw, L., 2022. Anthelmintic and antimycobacterial activity of fractions and compounds isolated from *Cissampelos mucronata*. J. Ethnopharmacol. 292, 115130.
Akande, R., Koma, O.S., Ibrahim, I., Efiom, O.O., 2013. Chemical constituents and antituberculosis activity of the root extracts of *Cissampelos owariensis* (P. Beauv.) Menispermaceae. Afr. J. Pure Appl. Chem. 7, 21–30.
Alicia, G., Gillian, C., 2017. Flowering Plants of Africa. South African National Biodiversity Institute.
Aro, A.O., Dzoyem, J.P., Hlokwe, T.M., Madoroba, E., Eloff, J.N., McGaw, L.J., 2015. Some South African Rubiaceae tree leaf extracts have antimycobacterial activity against pathogenic and non–pathogenic *Mycobacterium* species. Phytother. Res. 29, 1004–1010.

Arya, V., 2011. A review on anti-tubercular plants. Int. J. PharmTech Res. 3, 872–880.
Aska, A., Kubmarawa, D., 2016. Preliminary phytochemical screening of some indigenous medicinal plants used in the treatment of tuberculosis in Bauchi State, Nigeria. IOSR J. Appl. Chem. 9, 48–52.
Aska, A., Kubmarawa, D., Nkafamiya, I., Shagal, H., Oladosu, P., 2019. Quantitative phytochemical analysis and anti-tuberculosis activity of some selected medicinal plants in some northern parts of Bauchi state, Nigeria. IOSR 12, 15–22.
Bagla, V.P., McGaw, L.J., Elgorashi, E.E., Eloff, J.N., 2014. Antimicrobial activity, toxicity and selectivity index of two biflavonoids and a flavone isolated from *Podocarpus henkelii* (Podocarpaceae) leaves. BMC Complement. Altern. Med. 14, 1–6.
Barbosa-Filho, J., Da-Cunha, E.V.L., Gray, A.I., 2000. Alkaloids of the Menispermaceae. Alkaloids Chem. Biol. 54, 190.
Beesley, C.A., Vanner, C.L., Helsel, L.O., Gee, J.E., Hoffmaster, A.R., 2010. Identification and characterization of clinical *Bacillus* spp. isolates phenotypically similar to *Bacillus anthracis*. FEMS Microbiol. Lett. 313, 47–53.
Begue, W.J., Kline, R.M., 1972. The use of tetrazolium salts in bioautographic procedures. J. Chromatogr. A 64, 182–184.
Bhagya, N., Chandrashekar, K., Prabhu, A., Rekha, P., 2019. Tetrandrine isolated from *Cyclea peltata* induces cytotoxicity and apoptosis through ROS and caspase pathways in breast and pancreatic cancer cells. In Vitro. Cell. Dev. Biol. 55, 331–340.
Bhunu, B., Mautsa, R., Mukanganyama, S., 2017. Inhibition of biofilm formation in *Mycobacterium smegmatis* by *Parinari curatellifolia* leaf extracts. BMC Complement. Altern. Med. 17, 285.
Borges, A., José, H., Homem, V., Simões, M, 2020. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. Antibiotics 9, 48.
Bouquet, A., Debray, M. 1974. *Plantes medicinales de la Cote d'Ivoire.* Paris, ORSTOM. https://www.documentation.ird.fr/hor/fdi:06894 (accessed 12 November 2023).
Brown-Elliott, B.A., Philley, J.V., 2017. Rapidly growing mycobacteria. Microbiol. Spectr. 5, 1–19.
Bryskier, A., 2002. *Bacillus anthracis* and antibacterial agents. Clin. Microbiol. Infect. 8, 467–478.
Cavalcanti da Silva, E., Dias Rayol, C., Medeiros, P.L., Figueiredo, R.C., Piuvezan, M.R., Brabosa-Filho, J.M., Fernandes Marinho, A., Silva, T.G., Militão, G.C., Pimentel Cassilhas, A.P., Paes de Andrade, P., 2012. Antileishmanial activity of warifteine: a bisbenzylisoquinoline alkaloid isolated from Cissampelos sympodialis Eichl. (Menispermaceae). Sci. World J. 2012, 516408.
Darvin, S.S., Toppo, E., Esakkimuthu, S., Krishna, T.A., Ceasar, S.A., Stalin, A., Balakrishna, K., Muniappan, N., Pazhanivel, N., Mahaprabhu, R., 2018. Hepatoprotective effect of bisbenzylisoquinoline alkaloid tiliamosine from *Tiliacora racemosa* in high-fat diet/diethylnitrosamine-induced non-alcoholic steatohepatitis. Biomed. Pharmacother. 108, 963–973.
De Wet, H., Fouché, G., Van Heerden, F., 2009. *In vitro* cytotoxicity of crude alkaloidal extracts of South African Menispermaceae against three cancer cell linese. Afr. J. Biotechnol. 8.
De Wet, H., Struwig, M., Van Wyk, B.E., 2016. Taxonomic notes on the genera *Tiliacora* and *Tinospora* (Menispermaceae) in southern Africa. S. Afr. J. Bot. 103, 283–294.
De Wet, H., Van Heerden, F.R., Van Wyk, B.E., 2004. Alkaloids of *Antizoma angustifolia* (Menispermaceae). Biochem. Syst. Ecol. 32, 1145–1152.
De Wet, H., Van Wyk, B.E., 2008. An ethnobotanical survey of southern African Menispermaceae. S. Afr. J. Bot. 74, 2–9.
De Wet, H., 2005. An Ethnobotanical and chemotaxonomic study of South African Menispermaceae. PhD Thesis. University of Johannesburg, South Africa.
Earnest, E., Moke, G., Chinwuba, P., 2015. *Cissampelos owariensis*: experimental review. J. Pharm. Innov. 3, 75–77.
Famuyide, I.M., Aro, A.O., Fasina, F.O., Eloff, J.N., McGaw, L.J., 2019. Antibacterial and antibiofilm activity of acetone leaf extracts of nine under-investigated south African *Eugenia* and *Syzygium* (Myrtaceae) species and their selectivity indices. BMC Complement. Altern. Med. 19, 1–13.
Gandhi, N.R., Moll, A., Sturm, A.W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J., Friedland, G., 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet North Am. Ed. 368, 1575–1580.
González, L.T., Minsky, N.W., Espinosa, L.E.M., Aranda, R.S., Meseguer, J.P., Pérez, P.C, 2017. *In vitro* assessment of hepatoprotective agents against damage induced by acetaminophen and CCl 4. BMC Complement. Altern. Med. 17, 1–10.
Gunasekaran, H., Helan Chandra, J., Sridhar, S., 2016. Preliminary finger printing of chloroform, ethanol and aqueous root extracts of *Glycyrrhiza glabra* by comparing various mobile phase. J. Chem. Pharm. Sci. 9, 1434–1437.
Gupta, V.K., Kaushik, A., Chauhan, D.S., Ahirwar, R.K., Sharma, S., Bisht, D., 2018. Anti-mycobacterial activity of some medicinal plants used traditionally by tribes from Madhya Pradesh, India for treating tuberculosis related symptoms. J. Ethnopharmacol. 227, 113–120.
Ibekwe, N., Adelakun, T., Izebe, K., Adigwe, P., 2022. HPLC Fingerprinting and *in vitro* antimycobacterial activity of the roots of *Cissampelos owariensis* and *Cissampelos mucronata*. Trop. J. Nat. Prod. Res. (TJNPR) 6, 1249–1254.
Kahaliw, W., Aseffa, A., Abebe, M., Teferi, M., Engidawork, E., 2017. Evaluation of the antimycobacterial activity of crude extracts and solvent fractions of selected Ethiopian medicinal plants. BMC Complement. Altern. Med. 17, 1–9.
Kalscheuer, R., Palacios, A., Anso, I., Cifuente, J., Anguita, J., Jacobs, W.R., Guerin, M.E., Prados-Rosales, R., 2019. The *Mycobacterium tuberculosis* capsule: a cell structure with key implications in pathogenesis. Biochem. J. 476, 1995–2016.
Koenen, E.V., 1996. Medicinal, Poisonous and Edible Plants in Namibia. Klaus Hess Verlag, Göttingen, Germany.
Kokotkiewicz, A., Migas, P., Stefanowicz, J., Luczkiewicz, M., Krauze-Baranowska, M., 2017. Densitometric TLC analysis for the control of tropane and steroidal alkaloids in *Lycium barbarum*. Food Chem. 221, 535–540.
Kuete, V., 2010. Potential of Cameroonian plants and derived products against microbial infections: a review. Planta Med. 76, 1479–1491.
Kuete, V., Krusche, B., Youns, M., Voukeng, I., Fankam, A.G., Tankeo, S., Lacmata, S., Efferth, T., 2011. Cytotoxicity of some Cameroonian spices and selected medicinal plant extracts. J. Ethnopharmacol. 134, 803–812.
Lall, N., Kumar, V., Meyer, D., Gasa, N., Hamilton, C., Matsabisa, M., Oosthuizen, C., 2016. *In vitro* and *in vivo* antimycobacterial, hepatoprotective and immunomodulatory activity of *Euclea natalensis* and its mode of action. J. Ethnopharmacol. 194, 740–748.
Madikizela, B., Ndhlala, A.R., Finnie, J.F., Staden, J.V., 2013. *In vitro* antimicrobial activity of extracts from plants used traditionally in South Africa to treat tuberculosis and related symptoms. Evid. Based Complement. Altern. Med. 2013.
Mariita, R., Ogol, C., Oguge, N., Okemo, P., 2010. Antitubercular and phytochemical investigation of methanol extracts of medicinal plants used by the Samburu community in Kenya. Trop. J. Pharm. Res. 9.
McGaw, L., Lall, N., Meyer, J., Eloff, J., 2008. The potential of South African plants against *Mycobacterium* infections. J. Ethnopharmacol. 119, 482–500.
McGaw, L.J., Van Der Merwe, D., Eloff, J., 2007. *In vitro* anthelmintic, antibacterial and cytotoxic effects of extracts from plants used in South African ethnoveterinary medicine. Vet. J. 173, 366–372.
Molla, Y., Wubetu, M., Dessie, B., 2021. Anti-tuberculosis drug induced hepatotoxicity and associated factors among tuberculosis patients at selected hospitals, Ethiopia. Hepat. Med. 13, 1–8.
Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity. J. Immunol Methods 65, 55–63.
Muzila, M., Schmelzer, G.H., Gurib-Fakim, A., 2023. Cissampelos mucronata A. Rich. Plant Resources of Tropical Africa 11(1)- Medicinal plants 1. PROTA Foundation, Wageningen, Netherlands / Backhuys Publishers, Leiden, Netherlands / CTA, Wageningen, Netherlands.
Ndukwe, I., Amupitan, J., Isah, Y., Adegoke, K., 2007. Phytochemical and antimicrobial screening of the crude extracts from the root, stem bark and leaves of *Vitellaria paradoxa* (GAERTN. F). Afr. J. Biotechnol. 6.
Njeru, S.N., Obonyo, M.A., Nyambati, S.O., Ngari, S.M., 2015. Bioactivity of *Cissampelos pareira* medicinal plant against *Mycobacterium tuberculosis*. J. Pharmacogn. Phytochem. 3, 167–173.
Nondo, R.S., Mbwambo, Z.H., Kidukuli, A.W., Innocent, E.M., Mihale, M.J., Erasto, P., Moshi, M.J., 2011. Larvicidal, antimicrobial and brine shrimp activities of extracts from *Cissampelos mucronata* and *Tephrosia villosa* from coast region, Tanzania. BMC Complement. Altern. Med. 11, 1–7.
Okaiyeto, K., Nwodo, U., Mabinya, L., Okoh, A., 2018. A review on some medicinal plants with hepatoprotective effects. Pharmacogn. Rev. 12, 186.
Oliver-Bever, B., 1986. Medicinal Plants in Tropical West Africa. Cambridge university press, Cambridge, UK.
Oosthuizen, C.B., Gasa, N., Hamilton, C.J., Lall, N., 2019. Inhibition of mycothione disulphide reductase and mycobacterial biofilm by selected South African plants. S. Afr. J. Bot. 120, 291–297.
Priyadarshini, N., Veeramani, A., 2020. Antitubercular compounds isolated and characterized in *Tithonia diversifolia* and *Couroupita guianensis*. Int. J. Mycobacteriol. 9, 195.
Rahman, S.A., Singh, Y., Kohli, S., Ahmad, J., Ehtesham, N.Z., Tyagi, A.K., Hasnain, S.E., 2014. Comparative analyses of nonpathogenic, opportunistic, and totally pathogenic mycobacteria reveal genomic and biochemical variabilities and highlight the survival attributes of *Mycobacterium tuberculosis*. mBio 5, e02020–14.
Rukachaisirikul, T., Kumjun, S., Suebsakwong, P., Apiratikul, N., Suksamrarn, A., 2021. A new pyrrole alkaloid from the roots of *Cissampelos pareira*. Nat. Prod. Res. 35, 80–87.
Shai, L., Magano, S., Lebelo, S., Mogale, A., 2011. Inhibitory effects of five medicinal plants on rat alpha-glucosidase: comparison with their effects on yeast alpha-glucosidase. J. Med. Plants Res. 5, 2863–2867.
Shai, L.J., Masoko, P., Mokgotho, M.P., Magano, S.R., Mogale, A., Boaduo, N., Eloff, J.N., 2010. Yeast alpha glucosidase inhibitory and antioxidant activities of six medicinal plants collected in Phalaborwa, South Africa. S. Afr. J. Bot. 76, 465–470.
Sureram, S., Senadeera, S.P., Hongmanee, P., Mahidol, C., Ruchirawat, S., Kittakoop, P., 2012. Antimycobacterial activity of bisbenzylisoquinoline alkaloids from *Tiliacora triandra* against multidrug-resistant isolates of Mycobacterium tuberculosis. Bioorganic Med. Chem. Lett. 22, 2902–2905.
Tran, A.T., Watson, E.E., Pujari, V., Conroy, T., Dowman, L.J., Giltrap, A.M., Pang, A., Wong, W.R., Linington, R.G., Mahapatra, S., 2017. Sansanmycin natural product analogues as potent and selective anti-mycobacterials that inhibit lipid I biosynthesis. Nat. Commun. 8, 1–9.
Van Wyk, B.E., Gericke, N., 2000. People's plants: A guide to useful plants of southern Africa. Briza Publications, Pretoria.
POWO. 2023. Plants of the World Online. http://www.plantsoftheworldonline.org: Royal Botanic Gardens, Kew. (Accessed 23 October 2023).
Weber, C., Opatz, T., 2019. Bisbenzylisoquinoline alkaloids. Alkaloids Chem. Biol. 81, 1–114.
Wefferling, K.M., Hoot, S.B., Neves, S.S., 2013. Phylogeny and fruit evolution in Menispermaceae. Am. J. Bot. 100, 883–905.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Easthern Africa. 2nd Ed. Livingstone, Edinburgh & London, United Kingdom.
WHO 2019. Global tuberculosis report: executive summary. https://www.who.int/publications/i/item/9789241565714. (accessed 1 December 2023).
WHO 2022. Global tuberculosis report. https://www.who.int/publications/i/item/9789240061729. (accessed 1 December 2023).
Yang, N., He, J., Li, J., Zhong, Y., Song, Y., Chen, C., 2023. Predictors of death among TB/HIV co-infected patients on tuberculosis treatment in Sichuan, China: a retrospective cohort study. Medicine 102, e32811 (Baltimore)..