Flavonoids of *Chromolaena odorata* (L.) R.M.King & H.Rob. as potential leads for treatment against tuberculosis

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Highlights

- Flavonoids from *Chromolaena odorata* exhibited antimycobacterial activity against pathogenic *Mycobacterium bovis* and *M. tuberculosis* H37RV.
- Two flavonoids of *Chromolaena odorata* showed good hepatoprotective activity in HepG2 liver cells treated with rifampicin.
- Flavonoids of *Chromolaena odorata* showed a relatively safe margin.
- *Chromolaena odorata* compounds can serve as leads for the development of drugs against tuberculosis.

Abstract

Tuberculosis (TB) is currently rated as the 13th leading cause of mortality and the second leading cause of death after COVID-19, and above AIDS. Existing challenges relating to the development of multidrug-resistant strains and dangerous side effects of currently used drugs add impetus to the search for additional TB treatments. Hence, interest has grown in the use of medicinal plants as a source of bioactive preparations with efficacy against TB-causing organisms, and also with the ability to ameliorate the negative effects of TB drugs. This study aimed to evaluate the antimycobacterial and hepatoprotective potentials of extracts and isolated flavonoid compounds from invasive *Chromolaena odorata.* Test organisms used were pathogenic *Mycobacterium bovis* and *M. tuberculosis* H37RV, and the fast-growing *M. aurum, M. fortuitum* and *M. smegmatis*. The selectivity index (SI) values of the test substances were determined through cytotoxicity assays to promote these extracts and compounds as leads for the development of effective and safe anti-tubercular drugs. The antimycobacterial activity was evaluated using a serial microdilution method, and the SI was calculated from the 50% lethal concentrations calculated from cytotoxicity tests. Hepatoprotective activity was determined using HepG2 liver cells treated with rifampicin as a toxin. The extracts and compounds had a range of antimycobacterial activity with minimum inhibitory concentration (MIC) values ranging from 0.031 to 2.5 mg/mL. Two flavonoid compounds, 5,7,4′-trimethoxy flavanone and 5-hydroxy-3,7,4′-trimethoxyflavone showed promising antimycobacterial potential, and minimal toxicity was observed, as most SI values were higher than 1. The flavonoid compound 5,7,4′-trimethoxy flavanone had the highest SI (6.452), which was against *M. tuberculosis* H37RV. The HepG2 cells were reduced to 65% due to toxicity by rifampicin, however, the flavonoid compounds were able to improve cell viability to between 81 and 89% at different concentrations tested. Results obtained indicate that *C. odorata* may serve as a lead for the development of safe and effective antimycobacterial and hepatoprotective drugs.

Keywords: Tuberculosis; Antimycobacterial; Hepatoprotective; *Chromolaena odorata;* 5,7,4′-trimethoxy flavanone; 5-hydroxy-3,7,4′-trimethoxyflavone

1. Introduction

Tuberculosis (TB) is a highly contagious disease caused by either Mycobacterium tuberculosis or *M. bovis* in humans and animals respectively, and as a result, contributes to high morbidity and mortality rates globally (WHO, 2011; Bhargava and Bhargava, 2020; GHP, 2021). Its global threat cannot be overemphasized as it affects one-quarter of the world's population (WHO, 2021). Although TB infection may be latent in some people, it has the ability to cause the disease in the future (WHO, 2021). According to the World Health Organisation (WHO, 2021), TB is rated as the 13th leading cause of mortality and the second leading cause of death after COVID-19, above human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS). Due to the continuous threat of the disease to public health globally, the United Nations held a crucial meeting in 2018 to discuss and examine the progress that has been made globally, following a set goal to end the health crisis caused by TB by the year 2030 (GHP, 2021). According to a report by the Global Health Policy (GHP, 2021), about 10 million people develop active TB annually. Human Immunodeficiency Virus, which can break the natural defense mechanism in the body, has accelerated the outbreak of the disease (GHP, 2021; CDC, 2022). Reports have shown that almost two million lives are claimed by TB annually, and more than 25% of these cases are in Africa (WHO, 2011, 2022). In 2020 alone, deaths resulting from TB were 1.5 million, including about 214 000 people reported to be HIV-positive (GHP, 2021; WHO, 2021). A report by the WHO (WHO, 2021) indicated a decrease in the number of TB cases, however, it is still one of the leading causes of death.

Tuberculosis, which is mainly caused by *M. tuberculosis* in humans, can be transferred from person to person, however, zoonotic TB caused by *M. bovis* can also be transferred from animals to humans (WHO, 2018). The major means of transfer of *M. bovis* is the consumption of unpasteurized dairy products, but it can also be transferred by the consumption of uncooked meat or direct physical contact with animals (WHO, 2018). The presence of human *M. tuberculosis* has also been reported in animals (Atkins, 2000; de la Rua Domenech, 2006; Bilal et al., 2010). In 2016, about 147 000 new cases of zoonotic TB with 12 500 deaths were recorded, and most of these cases were reported in Africa (WHO, 2018). Although the cases of zoonotic TB caused by *M. bovis* only add a small fraction to the overall human TB disease burden, the goal set by the WHO to completely eradicate TB by 2030 will be an impossible mission without combating zoonotic TB (WHO, 2018).

A few antimycobacterial drugs are available, which include isoniazid, rifampicin, ethambutol, pyrazinamide and streptomycin as the first line of treatment for the disease. A drug with the product name Myrin®-P Forte (Pfizer) is a fixed dose combination of ethambutol hydrochloride (275 mg), rifampicin (150 mg), isoniazid (75 mg) and pyrazinamide (400 mg) administered to patients for the intensive phase of two months. This is followed by continuous treatment with rifampicin and isoniazid for a period of four to six months (WHO, 2018). However, numerous side effects due to long-term treatment are a big health challenge (NTMG, 2014; Mayo Clinic, 2018). One major example is hepatotoxicity which has been reported in different studies using experimental animals (Pal et al., 2006; Tostmann et al., 2008; Yue et al., 2009; Shih et al., 2013).

The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains has further complicated the situation (Bilal et al., 2010). Therefore, there is a continuous search for new lines of treatment that may be more effective and achieve better results in a shorter period with lower side effects. Natural sources such as plants have been reported as one of these promising perspectives (Cantrell et al., 2001; Okunade et al., 2004; Luo et al., 2011; Soejarto et al., 2012; Gechev et al., 2014; Dzoyem et al., 2016; Madikizela et al., 2017).

Chromolaena odorata (L.) R.M.King & H.Rob. is an alien invasive plant in South Africa with no history of traditional medicinal use. However, another biotype of *C. odorata* known as the Asian/West African biotype (AWAB) has been reported to be widely used as a source of medicines in Africa (Omokhua et al., 2016). Due to the antimycobacterial activity displayed by this plant as reported in a previous study (Omokhua et al., 2018) against the fast-growing non-pathogenic *Mycobacterium* strains, a further step was taken to evaluate the antimycobacterial activity of extracts and its isolated compounds against the pathogenic *M. tuberculosis* (H37Rv) and *M. bovis* and compare activity to the non-pathogenic ones. A further step was to evaluate the compounds for hepatoprotective activity, to determine their ability to reduce hepatic injuries caused by currently used TB drugs.

2. Materials and methods

2.1. Mycobacterial strains

Three non-pathogenic and two pathogenic strains were used in this study, namely Mycobacterium smegmatis (ATCC 1441), *M. aurum* (NCTC 10437), *M. fortuitum* (ATCC 6841), *M. bovis* (ATCC 27290) and *M. tuberculosis* H37RV (ATCC 27294).

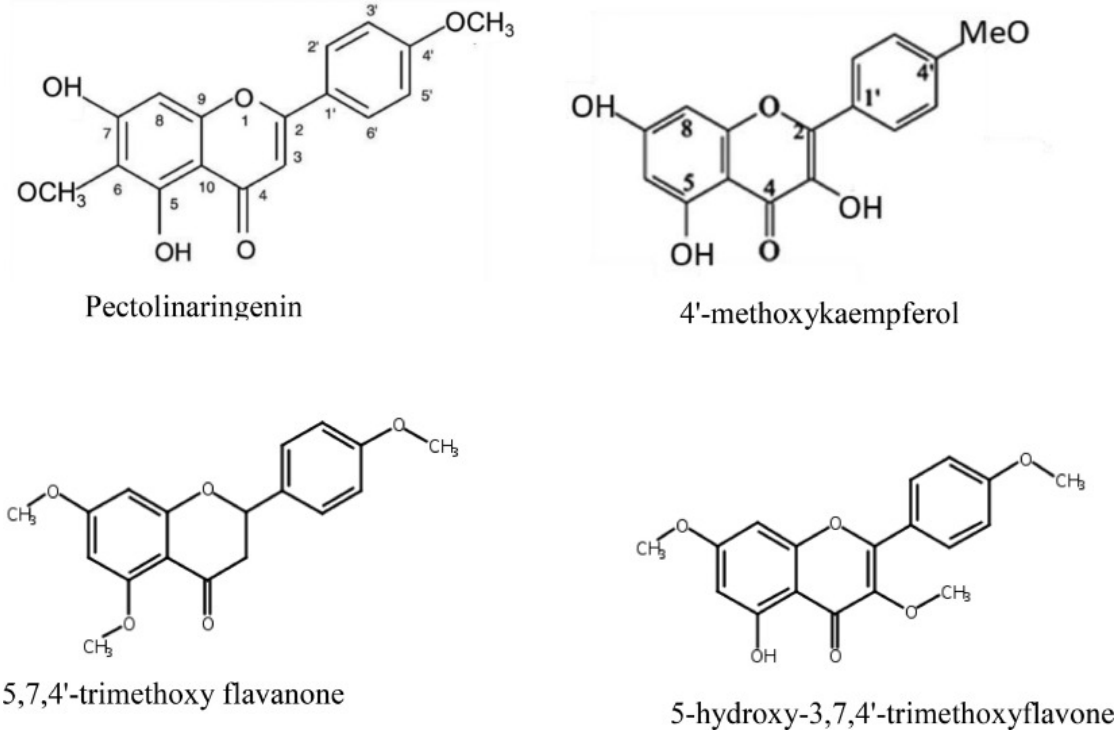

Fig. 1. Flavonoid compounds used in this study.

2.2. Plant collection, sample preparation and isolation

Plant extracts used for the study were acetone, dichloromethane and hot water extracts prepared following the procedure detailed in a previous study (Omokhua et al., 2018) while compounds isolated from a previous study (Omokhua-Uyi et al., 2020), (Fig. 1), namely pectolinaringenin, 4′-methoxykaempferol, 5,7,4′-trimethoxy flavanone and 5-hydroxy-3,7,4′-trimethoxyflavone, were used in this study.

2.3. Antimycobacterial activity

The pathogenic *Mycobacterium* strains under study were maintained on Lowenstein-Jensen (LJ) slants supplemented with glycerol except for *M. bovis*, where pyruvate was added, and both strains were cultured for one month. The non-pathogenic strains were maintained on Middlebrook 7H10 agar supplemented with 10% OADC and cultured for 24 h except for *M. aurum* which was cultured for 72 h. Cultured colonies were diluted in OADC-supplemented Middlebrook 7H9 broth, adjusted to McFarland standard No 1 (CFU/mL = 3.0×10^8), and diluted to a final density of 5×10^5 CFU/mL.

The 96-well microdilution method described by Eloff (1998) and Jadaun et al. (2007) was used to determine the antimycobacterial activity. A concentration of 10 mg/mL was prepared for the plant extracts using 10% dimethyl sulfoxide (DMSO) for the organic extracts and distilled water for hot water extracts. For the isolated compounds, 1 mg/mL solutions were prepared in 10% DMSO while 4 mg/mL of the positive controls were prepared using distilled water. To the wells of the sterile 96-well microplate, 100 µL of OADC-supplemented Middlebrook 7H9 broth were added, followed by the addition of 100 µL of the samples to the first wells (row A). A two-fold serial dilution was prepared down the wells of the microplate and 100 µL was discarded from the last wells. Streptomycin and rifampicin were used as positive controls, and 10% DMSO, water, inoculum and OADC-supplemented Middlebrook 7H9 broth as negative controls were also included. To all wells, 100 µL of mycobacterial cultures were introduced. To prevent evaporation, plates were sealed with parafilm and incubated at 37 °C for 24 h for the non-pathogenic strains and 7–10 days for the pathogenic strains. After the incubation period, 40 µL of 0.2 mg/mL of freshly prepared *p*-iodonitro-tetrazolium chloride (INT) solution was added to each well to determine the minimum inhibitory concentration (MIC). color detection after the addition of INT was read as soon as color became visible in the untreated control wells. The MIC values were read as the concentrations where a marked reduction in color formation corresponding to inhibition of mycobacterial growth was noticed. The prepared concentrations were tested in triplicate and the experiments were repeated twice.

2.4. Cytotoxicity screening and selectivity index

Cytotoxicity screening of the extracts and compounds was carried out in previous studies (Omokhua et al., 2018; Omokhua-Uyi et al., 2020) against mammalian cell lines including Vero monkey kidney, Caco-2 and C3A. The lethal concentration (LC_{50}) values of the compounds and extracts were determined in our previous studies (Omokhua et al., 2018; Omokhua-Uyi et al., 2020) as well as MIC values of the extracts against the non-pathogenic strains and these were used to calculate the selectivity index (SI) values. This was done to determine if the activity displayed by the compounds and extracts was due to toxicity against the mammalian cells which were investigated using the MTT assay. The human colon cell line Caco-2 was included in the study as the colon also plays an important role in drug

metabolism. Correlations between the MIC values of the pathogenic and non-pathogenic *Mycobacterium* were calculated using Microsoft Excel 2010 software.

2.5. Hepatoprotective activity

The hepatoprotective effect of the compounds was determined using HepG2 liver cells treated with rifampicin as a toxin according to González et al. (2017). The liver cells (HepG2) (2×10^4) were seeded in 100 µL Dulbecco's Modified Eagle Medium (DMEM) in a 96-well microplate. The plates were incubated for 24 h at 37 °C and 5% CO_2. The cells were treated with the toxicant (rifampicin, 1 mg/mL), and the compounds and silymarin (used as positive control) were prepared at different concentrations (0.0031 – 0.1 mg/mL) 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. After incubation, the media was removed and the cells were washed with phosphate-buffered saline (PBS) (200 µL), and 100 µL of media was added. MTT (5 mg/mL in PBS; 30 µL) was added to the cells and incubated for 2 h at 37 °C in a 5% CO_2 incubator. The medium was removed, and 50 µL DMSO was added to dissolve the MTT formazan crystals. The absorbance was read at 570 nm, and percentage cell viability was calculated.

3. Results

3.1. Antimycobacterial activity

The antimycobacterial activity displayed by the plant extracts and isolated compounds against pathogenic and non-pathogenic *Mycobacterium* strains is presented in Table 1. A wide range of antimycobacterial activity from good to weak activity was noted among the four compounds tested. However, only 5,7,4′-trimethoxy flavanone and 5-hydroxy-3,7,4′-trimethoxyflavone displayed good activity against both pathogenic and non-pathogenic strains. All plant extracts exhibited weak activity against the pathogenic strains in this study.

Table 1. Antimycobacterial activity of extracts and isolated compounds against pathogenic and non-pathogenic *Mycobacterium* strains.

Sample	Tested strains MIC (mg/mL)				
	M. bovis	***M. tb* H37Rv**	***M. aurum***	***M. fortuitum***	***M. smegmatis***
Pectolinaringenin	0.125	0.250	NA	NA	>0.25
4′-methoxykaempferol	0.125	0.125	NA	NA	0.250
5,7,4′-trimethoxy flavanone	**0.063***	**0.031****	NA	**0.063***	**0.063***
5-hydroxy-3,7,4′-trimethoxyflavone	**0.063***	**0.063***	NA	**0.063***	**0.063***
DCM	0.625	0.625	0.313	0.313	**0.156**
Acetone	0.313	2.500	0.313	0.313	0.313
Hot water	1.250	0.625	2.500	1.250	0.625
Strep (+ve con)	0.004	2.4×10^{-4}	0.004	>1	3.9×10^{-3}
Rif (+ve con)	0.004	2.4×10^{-4}	0.004	0.063	3.9×10^{-3}

Rif = rifampicin; Strep = streptomycin; +ve con = positive control; DCM = dichloromethane extract; *M. bovis = Mycobacterium bovis; M. tb = Mycobacterium tuberculosis; M. aurum = Mycobacterium aurum; M. fortuitum = Mycobacterium fortuitum; M. smegmatis = Mycobacterium smegmatis*; NA = not available; MIC = minimum inhibitory concentration. Values in bold indicate good activity; * = very good activity; ** = excellent activity.

3.2. Cytotoxicity and selectivity index values

Tables 2, 3 and 4 represents the SI values of the compounds and extracts which were calculated by dividing the LC_{50} of the samples obtained from cytotoxicity assays from previous studies (Omokhua et al., 2018; Omokhua-Uyi et al., 2020) with the MIC values observed. For C3A cells, the LC_{50} calculated was greater than 0.2 mg/mL, which was the highest concentration of the samples tested for toxicity assay, indicating no toxicity. But for the sake of calculating the SI values, an LC_{50} of 0.2 mg/mL was used. Comparing the SI calculated between the Vero, Caco-2 and C3A cells, the SI values were higher for most of the strains against the mammalian cells. The flavonoid 5,7,4′-trimethoxy flavanone had the highest SI (6.452) for all three cells against *M. tuberculosis* H37RV among the compounds tested.

Table 2. Selectivity index values of compounds and extracts tested against Vero monkey kidney cells.

Sample	Selectivity index LC_{50}/MIC				
	M. bovis	***M. tb* H37RV**	***M. aurum***	***M. fortuitum***	***M. smegmatis***
Pectolinaringenin	NA	NA	NA	NA	NA
4′-methoxykaempferol	1.60	1.60	NA	NA	0.80
5,7,4′-trimethoxy flavanone	3.206	**6.452**	NA	3.206	3.206
5-hydroxy-3,7,4′-trimethoxyflavone	3.127	3.127	NA	3.127	3.127
DCM	0.512	0.512	1.022	1.022	2.051
Acetone	2.204	0.276	2.204	2.204	2.204
Hot water	0.496	0.992	0.248	0.496	0.992
Strep (+ve con)	ND	ND	ND	ND	ND
Rif (+ve con)	**23.500**	**>100**	**23.500**	1.492	**24.103**

M. bovis = Mycobacterium bovis; M. tb = Mycobacterium tuberculosis; M. aurum = Mycobacterium aurum; M. fortuitum = Mycobacterium fortuitum; M. smegmatis = Mycobacterium smegmatis; ND = not detected; NA = not available; Strep = streptomycin; Rif = rifampicin; +ve con = positive control; MIC = minimum inhibitory concentration; LC_{50} = lethal concentration at 50%. Values written in bold showed the highest selectivity index indicating a relatively safe margin.

Table 3. Selectivity index values of compounds and extracts tested against Caco-2 cells.

Sample	Selectivity index LC_{50}/MIC				
	M. bovis	***M. tb* H37RV**	***M. aurum***	***M. fortuitum***	***M. smegmatis***
Pectolinaringenin	NA	NA	NA	NA	NA
4′-methoxykaempferol	1.54	1.54	NA	NA	0.772
5,7,4′-trimethoxy flavanone	3.175	**6.452**	NA	3.175	3.175
5-hydroxy-3,7,4′-trimethoxyflavone	2.524	2.524	NA	2.524	2.524
DCM	0.323	0.323	0.645	0.645	1.295
Acetone	0.358	0.045	0.358	0.358	0.358
Hot water	0.222	0.443	0.111	0.223	0.443
Strep (+ve con)	NA	NA	NA	NA	NA
Rif (+ve con)	NA	NA	NA	NA	NA

M. bovis = Mycobacterium bovis; M. tb = Mycobacterium tuberculosis; M. aurum = Mycobacterium aurum; M. fortuitum = Mycobacterium fortuitum; M. smegmatis = Mycobacterium smegmatis; ND = not detected; NA = not available; Strep = streptomycin; Rif = rifampicin; +ve con = positive control; MIC = minimum

inhibitory concentration; LC_{50} = lethal concentration at 50%. Values written in bold showed the highest selectivity index indicating a relatively safe margin.

Table 4. Selectivity index values of compounds and extracts tested against C3A cells.

Sample	Selectivity index LC_{50}/MIC				
	M. bovis	***M. tb* H37RV**	***M. aurum***	***M. fortuitum***	***M. smegmatis***
5,7,4′-trimethoxy flavanone	3.174	**6.452**	NA	3.174	3.174
5-hydroxy-3,7,4′-trimethoxyflavone	3.174	3.174	NA	3.174	3.174
DCM	0.323	0.323	0.639	0.639	1.282
Acetone	0.639	0.08	0.639	0.639	0.639
Hot water	0.16	0.323	0.08	0.16	0.625
Strep (+ve con)	NA	NA	NA	NA	NA
Rif (+ve con)	NA	NA	NA	NA	NA

M. bovis = Mycobacterium bovis; M. tb = Mycobacterium tuberculosis; M. aurum = Mycobacterium aurum; M. fortuitum = Mycobacterium fortuitum; M. smegmatis = Mycobacterium smegmatis; ND = not detected; NA = not available; Strep = streptomycin; Rif = rifampicin; +ve con = positive control; MIC = minimum inhibitory concentration; LC_{50} = lethal concentration at 50%. Values written in bold showed the highest selectivity index indicating a relatively safe margin.

3.3. Correlation between investigated non-pathogenic and pathogenic mycobacterium strains

Correlation coefficients observed among the pathogenic and non-pathogenic *Mycobacterium* strains investigated are presented in Fig. 2. Among the fast-growing non-pathogenic strains compared to pathogenic *M. bovis,* no correlation was observed in their antimycobacterial activity. A correlation was observed between *M. aurum* and *M. tuberculosis* (correlation coefficient = 0.1842) (Fig. 2c), however, this is regarded as weak as the value was below 1.

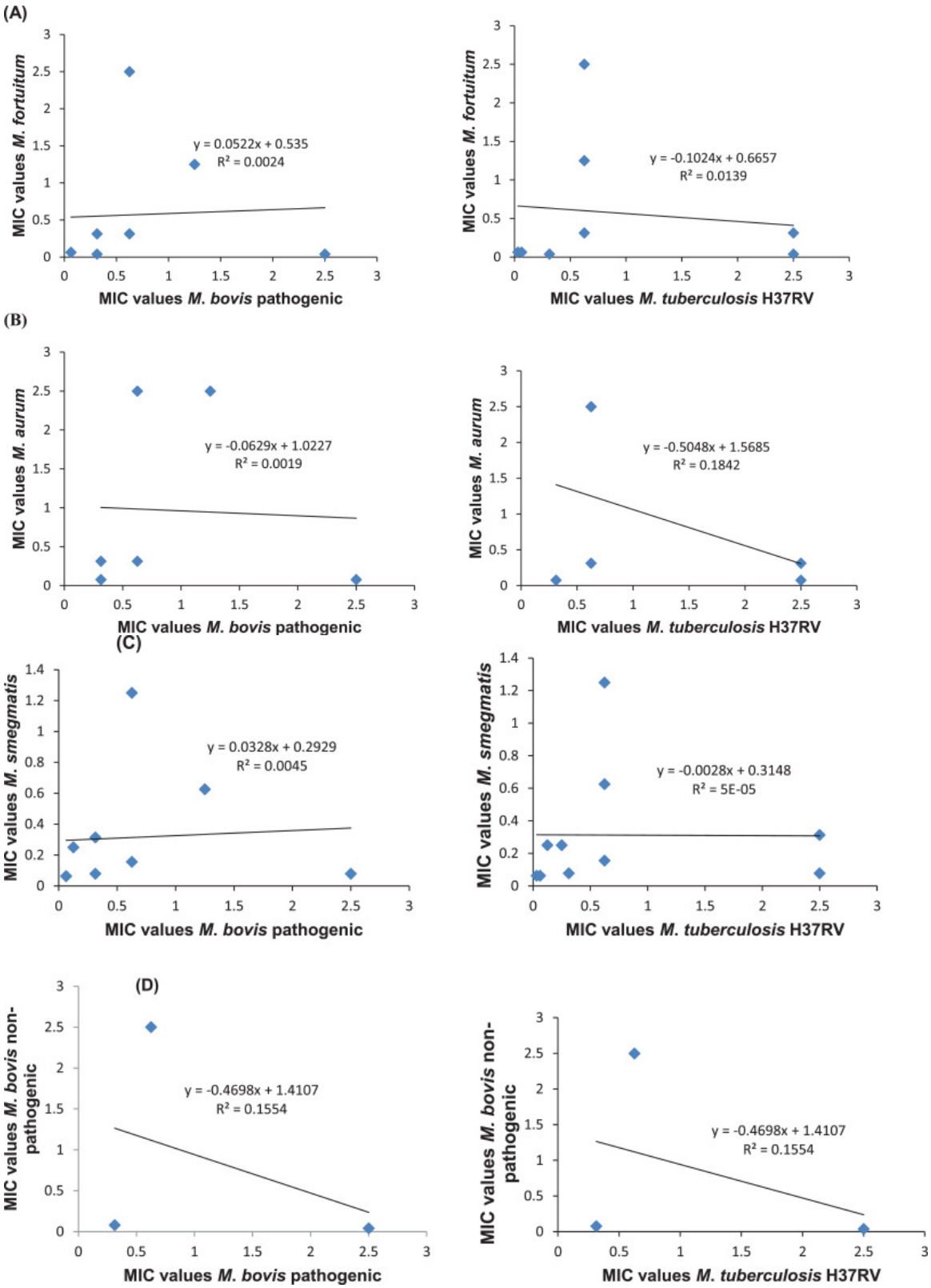

Fig. 2. Determination of correlation between the antimycobacterial activity of the non-pathogenic and pathogenic strains.

3.4. Hepatoprotective activity

Table 5 shows the hepatoprotective activity of the compounds and silymarin tested on HepG2 cells treated with rifampicin. When the HepG2 cells were treated with rifampicin, the cell viability was reduced to 65% due to toxicity. However, the flavonoid compounds were able **to improve the cell viability to between 81 and 89% at different concentrations tested.**

Table 5. Percentage hepatoprotective activity of two isolated compounds on HepG2 cells treated with rifampicin as a toxicant (mg/mL).

Concentration	5,7,4′-trimethoxy flavanone	5-hydroxy-3,7,4′-trimethoxyflavone	Silymarin	Rifampicin
1	–	–	–	65.26869±0.184
0.1	**86.448±0.0585**	**89.277±0.023**	**84.824±0.066**	–
0.05	81.793±0.0786	83.765±0.035	80.887±0.083	–
0.025	**85.731±0.062**	**84.085±0.0343**	79.821±0.087	–
0.0125	83.117±0.0728	83.571±0.0354	77.842±0.096	–
0.00625	**85.674±0.0618**	83.571±0.036	79.577±0.088	–
0.0031	82.044±0.0875	82.7138±0.037	79.368±0.091	–

Values in bold indicate a significantly higher percentage of hepatoprotective activity.

4. Discussion

In this study, weak antimycobacterial activity was exhibited by the compounds pectolinaringenin and 4′-methoxykaempferol against the pathogenic and non-pathogenic strains, while no activity was observed with pectolinaringenin against *M. smegmatis*. Good activity was only exhibited by the compounds 5,7,4′-trimethoxy flavanone and 5-hydroxy-3,7,4′-trimethoxyflavone against both the pathogenic and non-pathogenic strains. This same trend was observed with these compounds concerning the antimicrobial activity against uropathogenic organisms such as *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Aspergillus fumigatus* and *Cryptococcus neoformans* in a previous study (Omokhua-Uyi et al., 2020). This confirms the good antimicrobial properties of these two flavonoid compounds. Macedo et al. (2019) also reported the antimicrobial activity of 5-hydroxy-3,7,4′-trimethoxyflavone against multidrug-resistant (MDR) *S. aureus* and *E. coli*. To the best of our knowledge, this is the first study to report the antimycobacterial activity of the compound 5,7,4′- trimethoxy flavanone. Although reports on the antimycobacterial activity of the compound 5,7,4′- trimethoxy flavanone are scarce, Murillo et al. (2003) reported the antimycobacterial activity of the compound 5-hydroxy-3,7,4′-trimethoxyflavone isolated from an Asteraceae plant, *Haplopappus sonorensis* (A. Gray) S.F. Blake, against *M. tuberculosis* H37RV. This report affirms the findings from the present study.

Flavonoids in general have been reported to exhibit good activity against tuberculosis. For example, Favela-Hernández et al. (2012) reported that 5,4-dihydroxy-3,7,8,3-tetramethoxyflavone and 5,4-dihydroxy 3,7,8-trimethoxyflavone from the leaves of *Larrea tridentata* (DC.) Coville (Zygophyllaceae) were active against multidrug-resistant TB. Flavones such as artocarpin and chaplashin from the plant *Artocarpus altilis* (Parkinson) Fosberg (Moraceae), displayed antimycobacterial activity with MIC of 0.003 mg/mL, even better than the standard drug kanamycin (Boonphong et al., 2007). Studies have shown that flavonoids have the ability to interrupt specific mycobacterial mechanisms such as the disruption of nucleic acid and mycolic acid synthesis, which are essential for the survival of

the pathogen (Boonphong et al., 2007; Gygli et al., 2017). Hence, flavonoid compounds hold great potential in the development of antitubercular drugs.

In our previous study (Omokhua et al., 2018) where activity against the fast-growing non-pathogenic strains (*M. aurum, M. fortuitum* and *M. smegmatis*) was investigated against the plant extracts, only the dichloromethane (DCM) extract showed moderate activity (MIC = 0.156 mg/mL) which was against *M. smegmatis*. In this study, it is interesting to note that the plant extracts had weak activity across the table, whereas the compounds isolated from the plant showed good activity against the pathogenic and non-pathogenic strains (Table 1).

In the determination of the correlation between the non-pathogenic and the pathogenic mycobacterial strains, no strong correlations were found between the activity of the samples against the non-pathogenic and pathogenic *Mycobacterium* species used for the study.

The SI values calculated for all strains tested showed that the antimycobacterial activity displayed by the plant extracts and isolated compounds was not because of the toxicity of the samples against the Vero monkey kidney, C3A or Caco-2 cells. This indicates that the investigated extracts and especially the compounds isolated from *C. odorata* can potentially be useful as leads for the development of novel agents in the treatment of TB.

Medicinal plants have been reported to possess hepatoprotective effects against liver toxicity. For example, Thiesen et al. (2017) evaluated the hepatoprotective effect of the methanolic extract (300 µg/mL) from *Maytenus robusta* Reissek leaves in carbon tetrachloride (CCl_4)-induced hepatotoxicity in HepG2 cells. The extract was observed to restore cell viability from 29.56% to 65.27%. In this study, from our results obtained from the hepatoprotective activity, 5,7,4′-trimethoxy flavanone improved cell viability from 65% to 85.6% at a concentration of 0.00625 mg/mL, higher than the activity exhibited by silymarin. Even at 0.1 mg/mL, the percentage hepatoprotective effect exhibited by the flavonoid 5-hydroxy-3,7,4′-trimethoxyflavone (89.2%) was higher than that of silymarin (84.8%). Following the results displayed in Table 4, it can be calculated that these two flavonoids isolated from the South African invasive *C. odorata* plant have the potential to protect against hepatic injury or improve the ability of liver tissue to heal. Hence, these compounds can serve as leads for the development of hepatoprotective drugs, or as preparations to administer simultaneously with TB drugs to reduce their hepatotoxicity. To the best of our knowledge, this is the first study to report the antimycobacterial and hepatoprotective activities of the flavonoid compounds 5-hydroxy-3,7,4′-trimethoxyflavone and 5,7,4′-trimethoxy flavanone. Flavonoids have been reported by several authors to exhibit hepatoprotective effects. For example, in a study by Kim et al. (2011), flavonoids such as quercetin, hirsutrin and avicularin isolated from *Lespedeza cuneata* (Dum.-Cours.) G. Don. exhibited significant hepatoprotective activity against tert-butyl hyperoxide induced-toxicity on HepG2 cells. The ethanol extract of the plant was also very effective against liver toxicity. In another study, a flavonoid from *Glycyrrhiza uralensis* Fisch was able to repair liver tissues and reduce liver injury through the alleviation of inflammation and reducing oxidative stress (Gou et al., 2020). Ma et al. (2016) evaluated the hepatoprotective ability of flavonoids from *Cirsium japonicum* DC. against CCL_4-induced hepatocyte injury. The authors reported that the flavonoids were able to increase cell viability by reducing toxicity, and the activity displayed was similar to silymarin, which is used as an established hepatoprotective drug. No hepatoprotective activity studies have been carried out on any of the biotypes of *C. odorata*, except for the present study that focused on two isolated compounds. Some plants of the Asteraceae family have been reported to exhibit hepatoprotective ability in animal studies. For example,

Elephantopus scaber L. improved the liver biochemical changes in rats intoxicated by CCL_4 (Rajesh and Latha, 2001). An Asteraceae plant, *Coreopsis tinctoria* Nutt., used in traditional medicine for the treatment of hepatitis, exhibited a hepatoprotective effect against CCL_4 acute liver injury in rats. Its ability to reduce liver lesions was similar to the activity displayed by silymarin (Tsai et al., 2017).

5. Conclusions

For the first time the antimycobacterial and hepatoprotective activities of the extracts and compounds of *C. odorata* against *M. bovis* and *M. tuberculosis* has been reported. The results from the present study on the antimycobacterial activity of the extracts of this plant and isolated compounds have shown that this weed species has promising antimycobacterial properties. The antimycobacterial and hepatoprotective activities displayed by the flavonoid compounds 5,7,4′-trimethoxy flavanone and 5-hydroxy-3,7,4′-trimethoxyflavone from *C. odorata* against the pathogenic and non-pathogenic mycobacterial strains, and absence of cytotoxicity at concentrations tested has provided potential leads for the development of antimycobacterial agents for the treatment of TB. The ability of a drug target to be effective against TB and at the same time reduce hepatotoxicity is very important in the development of antitubercular drugs. The two flavonoid compounds were shown to exhibit such characteristics. Further studies including the use of animal models and clinical trials will be of utmost importance.

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.

Acknowledgement

AG Omokhua-Uyi (AG Omokhua) is grateful to the National Research Foundation-The World Academy of Science (NRF-TWAS) for providing funding.

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