

# Antibacterial activity of two biflavonoids from *Garcinia livingstonei* leaves against *Mycobacterium smegmatis*

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## Abstract

Amentoflavone and 4' monomethoxy amentoflavone were previously isolated from *Garcinia livingstonei* leaves. These compounds had good activities (MIC 6 and 8 µg/ml) against some nosocomial bacteria. In this study, the activity of these purified compounds were tested against fast-growing non-pathogenic *Mycobacterium smegmatis*. Amentoflavone was the most active compound, with an MIC of  $0.60 \pm 0.70$  mg/ml. The MIC of 4' monomethoxy amentoflavone and the positive control isoniazid against *Mycobacterium smegmatis* were similar  $1.40 \pm 1.56$  and  $1.30 \pm 1.70$  mg/ml respectively. Although, *Mycobacterium smegmatis* is a non-pathogenic fast growing mycobacterium the activities of these compounds may also be useful in combating infections by pathogenic *Mycobacterium* spp.

**Keywords:** Amentoflavone; 4' monomethoxy amentoflavone; Antimycobacterial activity; Tuberculosis

## 1. Introduction

Plant-derived compounds are a potential source for investigation of alternative lead chemical structures for drug development (McGaw et al., 2008). Several recent reviews emphasize the potential of plant species and natural products as sources of antimycobacterial extracts and chemicals (Newton et al., 2000, Asres et al., 2001, Cantrell et al., 2001 and Okunade et al., 2004). The structural diversity of plant-derived antimycobacterial compounds is highlighted by the fact that the classes to which these compounds belong include alkaloids, terpenoids, coumarins/chromones, peptides and phenolics (McGaw et al., 2008). Also flavonoids have been documented to have good antimicrobial activities (Cowan, 1999). We have previously isolated and chemically characterized biflavonoids from a *Garcinia livingstonei* (Kaikabo et al., 2009). These compounds had excellent activity with MICs of 6 and 8 µg/ml against *Enterococcus faecalis* and *Escherichia coli* respectively. Because the plant from which these compounds were isolated have been used traditionally to treat respiratory ailments and tuberculosis is such an important disease, we decided to determine the activity of these biflavonoids against mycobacteria.

## 2. Materials and methods

### 2.1. Preparation of pure compounds

The pure compounds amentoflavone and 4-monomethoxy amentoflavone were isolated from a leaf extract of *Garcinia livingstonei* (Voucher ID: GL/K/2008) as previously described (Kaikabo et al., 2009). A stock solution of the pure compounds was prepared in DMSO to a concentration of 20 mg/ml.

### 2.2. Experimental procedures

#### 2.2.1. Mycobacterial culture

Antimycobacterial activity was tested against *Mycobacterium smegmatis* (ATCC 1441) as described by McGaw et al. (2008). It was cultured on Löwenstein–Jensen agar slants, supplemented with glycerol. Sterile plastic loops were used to scrape cells off the slants and these were carefully suspended in a small volume of sterile distilled water to avoid formation of clumps. The suspension was diluted with sterile water to render a concentration of cells equal to a MacFarland No. 1 standard solution (approximately  $4 \times 10^7$  cfu/ml), and then diluted with freshly prepared Middlebrook 7H9 broth supplemented with 10% OADC medium to obtain a final inoculum density of approximately  $5 \times 10^5$  cfu/ml. This was confirmed by spreading 100 µl volumes of

10-fold serial dilutions of each culture suspension onto agar plates using a glass spreader and counting colonies growing after incubation at 37 °C.

### 2.2.2. Antimycobacterial assay

A modified two-fold serial dilution assay in 96-well microtitre plates was used to detect antimycobacterial activity ( Seidel and Taylor, 2004 and McGaw et al., 2008 ). Isolated compounds were serially diluted (100 µl) with OADC supplemented Middlebrook 7H9 broth in wells of microtitre plates before mycobacterial culture (100 µl) was added to each well. The anti-tuberculosis drug isoniazid was used as a positive control at a concentration of 0.1 mg/ml, and solvent controls were included. Doses were tested in triplicate and the entire experiment was repeated, providing six data sets per dilution.

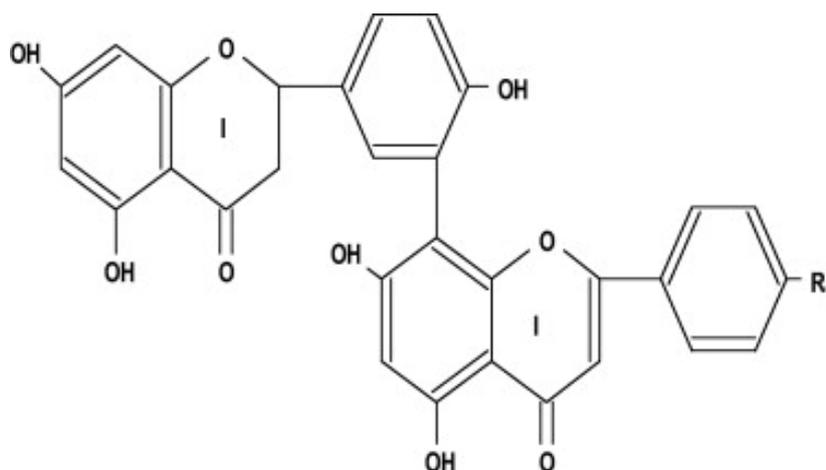
The microplates were sealed with parafilm and placed in a stainless steel chamber, the base of which was lined with paper towel saturated with sterile water to maintain humidity; this was incubated at 37 °C for 24 h. MIC values were detected using a tetrazolium violet (INT) indicator (Eloff, 1998). The colour reaction after addition of INT generally occurred after a 1 h incubation period. Minimal inhibitory concentration (MIC) values were read as those concentrations where a marked reduction in colour formation was noted.

## 3. Results

The antimycobacterial activity of the two compounds and the positive control is provided in **Table 1**. Amentoflavone with an MIC of  $0.60 \pm 0.70$  mg/ml was more active than 4' monomethoxyamentoflavone and INH having MIC values of  $1.40 \pm 1.56$  and  $1.30 \pm 1.70$  mg/ml respectively. The structure of the two biflavonoid compounds is shown in **Fig. 1** depicting biflavonoids.

**Table 1.** Antimycobacterial Activity of purified Compounds from a *Garcinia livingstonei* leaf extract (MIC in mg/ml).

Test samples	Minimum inhibitory concentration
Isoniazid (INH)	$1.30 \pm 1.70$
Amentoflavone	$0.60 \pm 0.70$
4' monomethoxyamentoflavone	$1.40 \pm 1.56$



**Fig. 1.** : The structure of the two compounds isolated from a *Garcinia livingstonei* leaf extract evaluated for antimycobacterial activity.

#### 4. Discussion

The broth dilution technique used in this study offers benefits such as, ease of operation, no expensive equipment required low sample volumes required in the microplate format. Additionally, in liquid medium there is increased cell-to-drug contact and the shorter incubation time than is required for agar methods lowers the likelihood of breakdown of the test compounds (Lall and Meyer, 1999).

Many scientists have used the 96-well microplate serial dilution method with tetrazolium salts to determine antibacterial activity developed by Eloff. Since then it has also been used to determine antifungal activity (Masoko et al., 2005) and drug susceptibility for mycobacteria (Gomez-Flores et al., 1995). The tetrazolium salt is converted to a coloured formazan salt in the presence of actively dividing cells. The technique is rapid, has low technology requirements, is inexpensive and uses a microplate assay that has the potential of becoming the method of choice for drug susceptibility testing of *Mycobacterium tuberculosis* in places where TB is a major problem (Franzblau et al., 1998). The same protocol with INT as indicator have been used in this study which has worked effectively.

Biflavonoids have good antibacterial activities against microbes (Cowan, 1999). Kaikabo et al. (2009) have reported MICs of 6 and 8 µg/ml of 4' methoxyamentoflavone against *Enterococcus faecalis* and *Escherichia coli* respectively. These results motivated the study of the antimycobacterial activity against *Mycobacterium smegmatis*.

Amentoflavone was more active than the conventional drug isoniazid employed in the clinical treatment of tuberculosis in humans and 4' monomethoxyamentoflavone had a similar activity. Replacement of the hydroxyl with a methoxy moiety decreased the antimycobacterial activity. This did however lead to a decrease in the cytotoxic concentration. Amentoflavone and 4' monomethoxyamentoflavone has cytotoxic concentrations killing 50% of the cells at 0.38 and >0.6 mg/mL respectively (Kaikabo et al., 2009). This indicates the possibility that the two compounds were relatively safe and that chemical modification may lead to a potentially useful compound.

The good antimycobacterial activities of the two biflavonoids compared to isoniazid and the relatively low cellular toxicities indicates that the two compounds were relatively safe and that chemical modification may lead to a potentially useful antituberculosis compound.

If the activity against *Mycobacterium smegmatis* is also apparent against *Mycobacterium tuberculosis* and *Mycobacterium bovis* and the relatively low cellular toxicity is reflected in animal toxicity studies preclinical trials of these compounds and derivatives could be warranted.

The observed activities of these compounds may explain why the natives use the extract of this plant in the treatment of respiratory related ailments.

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#### References

- K. Asres, F. Bucar, S. Edelsbrunner, T. Kartnig, G. Hö and W. Thiel, Investigations on Antimycobacterial activity of some Ethiopian medicinal plants. *Phytotherapy Research*, **15** (2001), pp. 323–326.
- C.L. Cantrell, S.G. Franzblau and N.H. Fischer, Antimycobacterial plant terpenoids. *Planta Medica*, **67** (2001), pp. 685–694.
- M.M. Cowan, Plant products as antimicrobial agents. *Clinical Microbiological Reviews*, **12** (1999), pp. 564–582.

J.N. Eloff, A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica*, **64** (1998), pp. 711–714.

S.G. Franzblau, R.S. Witzig, J.C. McLaughlin, P. Torres, G. Madico, A. Hernandez, M.T. Degnan, M.B. Cook, V.K. Quenzer, R.M. Ferguson and R.H. Gilman, Rapid, low-technology MIC determination with clinical *Mycobacterium tuberculosis* isolates by using the microplate Alamar Blue Assay. *Journal of Clinical Microbiology*, **36** (1998), pp. 362–366.

R. Gomez-Flores, S. Gupta, R. Tamez-Guerra and R.T. Mehta, Determination of MICs for *Mycobacterium avium*–*M. intracellulare* complex in liquid medium by a colorimetric method. *Journal of Clinical Microbiology*, **33** (1995), pp. 1842–1846.

A.A. Kaikabo, B.B. Samuel and J.N. Eloff, Isolation and activity of two antibacterial biflavonoids from *Garcinia livingstonei* leaf extract. *Natural Products Communications*, **4** (2009), pp. 1–4.

N. Lall and J.J.M.J. Meyer, In vitro inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. *Journal of Ethnopharmacology*, **66** (1999), pp. 347–354.

P. Masoko, J. Picard and J.N. Eloff, Antifungal activities of six South African *Terminalia* species (Combretaceae). *Journal of Ethnopharmacology*, **99** (2005), pp. 301–308.

L.J. McGaw, N. Lall, T.M. Hlokwé, A.L. Michel, J.J.M. Meyer and J.N. Eloff, Purified compounds and extracts from *Euclea* species with antimycobacterial activity against *Mycobacterium bovis* and fast-growing mycobacteria. *Biological Pharmaceutical Bulletin*, **31** (2008), pp. 1429–1433.

S.M. Newton, C. Lau and C.W. Wright, A review of antimycobacterial natural products. *Phytotherapy Research*, **14** (2000), pp. 303–322.

A.L. Okunade, M.P.F. Elvin-Lewis and W.H. Lewis, Natural antimycobacterial metabolites: current status. *Phytochemistry*, **65** (2004), pp. 1017–1032.

V. Seidel and P.W. Taylor, In vitro activity of extracts and constituents of *Pelargonium* against rapidly growing mycobacteria. *International Journal of Antimicrobial Agents*, **23** (2004), pp. 613–619.