Journal of Ethnopharmacology 156 (2014) 228–234

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Research paper

Isolation and characterization of antimicrobial compounds from *Terminalia phanerophlebia* Engl. & Diels leaf extracts

B. Madikizela, M.A. Aderogba, J.F. Finnie, J. Van Staden *

Research Centre for Plant Growth and Development, School of Life Sciences, University of KwaZulu-Natal, Scottsville 3209, Private Bag X01,Pietermaritzburg, South Africa

ARTICLE INFO

Article history:
Received 20 June 2014
Received in revised form
26 August 2014
Accepted 2 September 2014
Available online 16 September 2014

Keywords:
Antimicrobial
Methyl gallate
Phenylpropanoid glycoside
Terminalia phanerophlebia
Tuberculosis

ABSTRACT

Ethnopharmacological relevance: The emergence of drug resistant-tuberculosis and other pathogenic diseases over the past decades, constitutes a serious threat to human health worldwide. According to a 2012 report by the World Health Organization (WHO), South Africa, China, India and Russia are the countries with the highest prevalence of Multi-Drug Resistant tuberculosis (MDR-tuberculosis) as they represented 60% of the total. Several reports have documented antimycobacterial properties of *Terminalia* species but only a few species from this genus have been explored for their antimycobacterial constituents. The crude extracts of *Terminalia phanerophlebia* showed good antimicrobial activities in our previous study against two *Mycobacterium* as well as two other bacterial strains responsible for opportunistic infections related to respiratory ailments. This paper studies the isolation of compounds responsible for such activities and to isolate compounds responsible for antimicrobial activities from the crude extracts of *Terminalia phanerophlebia* leaves.

Materials and methods: Terminalia phanerophlebia crude extracts obtained from 80% methanol was successively extracted with hexane, dichloromethane (DCM), ethyl acetate (EtOAc) and n-butanol. The fractions obtained and isolated compounds were tested for their antibacterial activities against *Mycobacterium aurum*, *Mycobacterium tuberculosis*, *Staphylococcus aureus* and *Klebsiella pneumoniae*. Bioguided fractionation of the EtOAc fraction afforded two bioactive compounds. Structure elucidation was carried out using NMR (1D and 2D) spectroscopic methods.

Results: EtOAc fraction exhibited highest antimicrobial activities and its fractionation afforded methyl gallate (methyl-3,4,5-trihydroxybenzoate) (1) and a phenylpropanoid glucoside, 1,6-di-O-coumaroyl glucopyranoside (2) These compounds are reported from *Terminalia phanerophlebia* for the first time. Both compounds showed good antimicrobial activity against all bacterial strains tested with minimum inhibitory concentration (MIC) values ranging from 63 to 250 μg/mL. Inhibition of *Mycobacterium tuberculosis* by 1,6-di-O-coumaroyl glucopyranoside (2) at a MIC value of 63 μg/mL was noteworthy, as this bacterial strain is reported to be the leading cause of tuberculosis worldwide.

Conclusions: Good antimicrobial activities exhibited by the compounds isolated from *Terminalia phanerophlebia* authenticate the traditional use of this plant in treating tuberculosis and its related symptoms. Compound (2), 1,6-di-O-coumaroyl glucopyranoside could serve as a lead compound for tuberculosis drug discovery.

© 2014 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

The past two decades have seen the emergence of pathogenic infectious diseases such as acquired immunodeficiency syndrome (AIDS) and drug-resistant tuberculosis which represent a substantial global threat to human health (Chen et al., 2011). Tuberculosis is presently one of the most serious bacterial infectious diseases that cause a threat to health care of humans globally (Chen et al., 2011). It is most commonly caused by *Mycobacterium tuberculosis*, but non-tuberculous mycobacteria can also cause comparable manifestations when the host has a weak immune system or

Abbreviations: AIDS, acquired immunodeficiency syndrome; ATCC, American type culture collection; CFU, colony forming unit; DCM, dichloromethane; DMSO, dimethylsulfoxide; EtOAc, ethyl acetate; HIV, human immunodeficiency virus; INT, p-iodoni-trotetrazolium violet; MDR, multi-drug-resistant; MeOH, methanol; MH, Mueller-Hinton; MHz, Mega hertz; MIC, minimum inhibition concentration; NMR, nuclear magnetic resonance; No.1, number 1; OADC, oleic acid-albumin-dextrose-catalase; OD, optical density; ppm, part per million; REMA, resazurin microplate assay; TLC, thin layer chromatography; TM, traditional medicine; UKZN, University of KwaZulu-Natal; WHO, World Health Organization; XDR, extensive-drug resistant

* Corresponding author. Tel.: +27 33 2605130.
E-mail address: rcpgd@ukzn.ac.za (J. Van Staden).

http://dx.doi.org/10.1016/j.jep.2014.09.003
0378-8741/© 2014 Elsevier Ireland Ltd. All rights reserved.

exposed under high doses of the bacterium (World Health Organisation, 2013; Kumar et al., 2014). There has been an emergence of resistant tuberculosis strains, to first line antibiotics known, as single-drug resistant, Multi-Drug Resistant (MDR) and Extensive-Drug Resistant (XDR) tuberculosis (World Health Organisation, 2009). It is estimated by the World Health Organization (WHO) that 50 million people may be infected with drug resistant tuberculosis worldwide (World Health Organisation, 2009). In 2012, a report issued by the WHO stated that China, India, Russian Federation, and South Africa represents 60% of the total cases of MDR-tuberculosis, thus referred to as the burden countries out of 27 nations (Sotgiu et al., 2013). MDR-tuberculosis is widespread and is recognized as a threat to tuberculosis control as it may result in death unless treated quickly and effectively (Alexander and De, 2007). South Africa is listed among four countries estimated to have the leading number of XDR-tuberculosis (Fyhrquist et al., 2014). All South African provinces have reported XDR-tuberculosis cases, since the Tugela Ferry outbreak in September, 2006 (Olson et al., 2011). The disturbing epidemiology of MDR and XDR-tuberculosis strains and difficulty in treating the disease creates an urgent need for solving the problem. According to the WHO, this is a new serious global public health problem (De Souza, 2009). Reports from all over the world have shown that XDR-tuberculosis treatment outcomes are poor. It is likely that more resistant strains of *Mycobacterium* that will exhaust the current chemical defences that are available presently will emerge in the near future.

To counteract this problem, there is need to find new tuberculosis treatments as the current ones are no longer as effective. According to Gupta et al. (2014), diverse compounds with antimicrobial activity have been isolated from higher plants. Many of these compounds have shown promising potential to cure infectious diseases (Fyhrquist et al., 2014). Therefore, compounds that might act as effective drugs for the treatment of tuberculosis might be found in medicinal plants.

Terminalia phanerophlebia Engl. & Diels belongs to the family Combretaceae, under the genus *Terminalia*. Different parts (roots, barks and leaves) of *Terminalia phanerophlebia* are used in traditional medicine (TM) for treatment of various diseases such as: pneumonia, bilharzia, hypertension, cancer, diabetes, stomach problems, schistosomiasis, gonorrhea, syphilis, gynecological conditions, inflammation, epilepsy, sexual transmitted diseases, wounds and skin disease (Neuwinger, 1996; McGaw et al., 2008; Van Wyk et al., 2000). In South Africa, different cultures use roots of *Terminalia phanerophlebia* to treat witchcraft associated diseases that are believed to culminate in coughing leading to tuberculosis and rheumatism (Mabogo, 1990). The crude extracts of the leaves, twigs and roots of this plant have demonstrated good antimicrobial activities against bacterial strains known to cause tuberculosis and opportunistic infections of the respiratory tract. Three compounds were reported from its stem bark (Nair et al., 2012). The compounds were identified as β-sitosterol, β-sitostenone and stigmast-4-ene-3,6-dione. The study demonstrated β-sitosterol as an anti-inflammatory agent. Many reports have mentioned that *Terminalia* contains antimycobacterial properties, however only a few species have been investigated for their antimycobacterial constituents (McGaw et al. 2008; Fyhrquist et al., 2014). In a study by Green et al. (2010) the *Terminalia sericea* Burch. ex DC. stem bark acetone extract was proven to have antimycobacterial activities as it inhibited the growth of both the attenuated and a clinical strain of *Mycobacterium tuberculosis*. The leaf crude extracts of *Terminalia glaucescens* Planch. was reported to inhibit the growth of the revertant strain of *Mycobacterium tuberculosis* in a study by Nvau et al. (2011), it also showed inhibitory activities against other *Mycobacterium* species such as *Mycobacterium ulcerans*, *Mycobacterium madagascariense* and *Mycobacterium indicus*. Promising antimycobacterial effects were demonstrated by root extracts of *Terminalia avicennioides* Guill. and Perr. against two *Mycobacterium* species: *Mycobacterium tuberculosis* and *Mycobacterium bovis* (Fyhrquist et al., 2014). Antimycobacterial compounds have been isolated from species of *Terminalia*, thus proving that some of the species of this genus have antimycobacterial effects. Compounds such as 3,4′-di-O-methylellagic acid 3′-O-β-D-xylopyranoside and 4′-O-galloy-3,3′-di-O-methyllegic acid 4-O-β-D-xylopyranoside isolated from stem bark extract of *Terminalia superba* Engl. & Diels exhibited good antimycobacterial activities against four strains of mycobacteria (Kuete et al., 2010). Pentacyclic triterpenoid and fridelin isolated from the leaf extracts of *Terminalia avicennioides* demonstrated promising inhibitory activity against *Mycobacterium bovis* (Fyhrquist et al., 2014). Acetyl rhamnosides of 1.3-hydroxylated pentacyclic triterpernoids from *Terminalia stuhlmannii* Engl. showed good inhibition of *Mycobacterium fortuitum* (Mann et al., 2011). More studies on the isolation of antimycobacterial compounds from *Terminalia* species are needed as some of the plants from this genus have proven to have antimycobacterial constituents.

In our previous studies on *in vitro* antimicrobial and antimycobacterial, anti-inflammatory as well as genotoxicity evaluation of extracts from plants used traditionally in South Africa to treat tuberculosis and related symptoms, we reported several plant extracts that exhibited good antibacterial activities against *Mycobacterium* species and other bacterial strains associated with respiratory infections (Madikizela et al., 2013a, 2014). *Terminalia phanerophlebia* leaf extracts showed considerable antimicrobial activities against bacterial strains tested and exhibited non-genotoxicity against *Salmonella typhimurium* strains. This prompted bioguided fractionation of its leaf extracts to isolate and identify the active compound(s) from this plant. Thus, the aims of this study were to isolate and identify active antimicrobial compounds from *Terminalia phanerophlebia*.

2. Materials and methods

2.1. General

All thin layer chromatography analyses were performed at room temperature using pre-coated plates (MERCK, silica gel $60F_{254}$ 0.2 thickness). Detection of spots was done by viewing under ultraviolet light (254 and 366 nm). Open column chromatography was carried out using silica gel (230–400 mesh) and Sephadex LH-20. Nuclear magnetic resonance (NMR) data were obtained on Brucker spectrometer (500 MHz). Chemical shifts are expressed in parts per million (ppm).

2.2. Plant material –collection and authentication

The leaves of *Terminalia phanerophlebia* were collected on February, 2013 from the University of KwaZulu-Natal (UKZN) botanical garden, Pietermaritzburg, South Africa. The plant was identified by Allison Young (UKZN, Horticulturist) and the voucher specimen (BALUNGI 37) was deposited in the UKZN Herbarium (NU) (Pietermaritzburg, South Africa) for botanical authentication. The collected leaves were separated from stalks and oven dried at 50 °C. When completely dried, the leaves were ground into powder and kept in airtight containers until use.

2.2.1. Extraction

The powdered plant material (1 kg) was extracted with 8 L of 80% methanol (MeOH) at room temperature for 24 h with occasional shaking and filtered through Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure using a

Download English Version:

https://daneshyari.com/en/article/2545261

Download Persian Version:

https://daneshyari.com/article/2545261

Daneshyari.com