



Antimycobacterial activity against different pathogens and selectivity index of fourteen medicinal plants used in southern Africa to treat tuberculosis and respiratory ailments



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ABSTRACT

Many plants are used in traditional medicine to treat tuberculosis and other respiratory disorders in Africa. The emergence of multiple drug resistance has become a major threat and thus calls for an urgent need to search for new effective and safe anti-TB agents. The aim was to determine the antimycobacterial activity and the safety of the acetone leaf extracts of 14 plant species used in southern Africa to treat tuberculosis and pulmonary ailments.

The antimycobacterial activity was evaluated by a tetrazolium violet based broth microdilution method against three fast-growing mycobacteria species (*Mycobacterium smegmatis*, *Mycobacterium aurum* and *M. fortuitum*) and one pathogenic *M. tuberculosis* field strain. The *in vitro* cellular toxicity was determined using the MTT assay on Vero monkey kidney cells. The extraction yield, the LC₅₀ and MIC values were used to determine the total activity (TA) and the selectivity index (SI) of the extracts.

Extracts had moderate to weak activity with the MIC values ranging from 0.039 to >2.5 mg/mL. *M. fortuitum* appeared to be better predictor of activity against pathogenic *M. tuberculosis* than *M. smegmatis* and *M. aurum*. Extracts from *Heteropyxis natalensis* (3.3) and *Hexalobus monopetalus* (2.47) had the highest selectivity index. The results substantiate the safety and in some cases the potential efficacy of the traditional use of these species against tuberculosis and pulmonary ailments.

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1. Introduction

Tuberculosis (TB) is a life-threatening disease for both humans and animals caused by various *Mycobacterium* species and is a leading cause of human mortality in the developing world (Philips and Ernst, 2012). In 2012, approximately 8.6 million people suffered from TB and 1.3 million died from it, and there were an estimated 450 000 new cases of multidrug-resistant TB worldwide (World Health Organization, 2013). The emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis has become a major threat. Therefore, there is an urgent need to find new effective and safe anti-TB agents. Although rapidly growing mycobacteria are of low virulence and generally considered as weak pathogens compared to *M. tuberculosis*, many species are of emerging clinical importance, capable of causing a wide spectrum of infections, particularly in immune-compromised patients (Hazara et al., 2014). Therefore, new agents with enhanced activity against

mycobacteria are also needed. Medicinal plants are used in many parts of southern Africa to treat TB-related symptoms, including chest complaints and coughing (McGaw and Eloff, 2008). Furthermore, the initial steps in new drug discovery from natural sources involve *in vitro* activity testing of plant extracts against target pathogenic microorganisms (Katiyar et al., 2012). Chung et al. (1995) showed that activity against the rapidly growing *M. aurum* is a good predictor of activity against *M. tuberculosis*, as the two species have similar drug sensitivity profiles. We have determined the antimicrobial activity of acetone leaf extracts of more than 500 trees species against several microorganisms including *M. smegmatis* (Pauw and Eloff, 2014). We selected plants, mainly trees, which have been used traditionally against indications that could be related to tuberculosis. The primary aim of this study was not to confirm the traditional use of medicinal plants; therefore, we used only leaves, a sustainable resource and only acetone as extractant. Acetone is the best extractant for antimicrobial activity from several plant species (Eloff, 1998a), and water extracts have hardly any antimicrobial activity (Kotze and Eloff, 2002; Eloff et al., 2005). Consequently, acetone was used as an extractant and not water. If an extract has high antimicrobial activity and is more toxic to mammalian cells, it may not be useful. Consequently, we determined the cytotoxicity and selectivity indices of the different extracts. The

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aim of this paper was to determine which plants could yield possibly useful products. A secondary aim was to determine the correlation between activities of different *Mycobacterium* species in order to use the Phytomedicine Programme database to find plants with a potential to deliver useful products.

2. Materials and methods

The leaves of plants were collected in the Pretoria National Botanical Garden. The identity of the plant material was confirmed by the curator, and voucher specimens were placed in the HGWJ

Table 1
Characteristics of the fourteen medicinal plants investigated.

Plant name (Family)	Common names	Part used	Traditional use	Voucher number
<i>Hexalobus monopetalus</i> (A. Rich.) Engl. & Diels (Annonaceae)	Baboons' breakfast or Shakama plum (Eng.). Muhodzongwa, mukorongwa, mukwingiziri, munyani, mupodzongo, mupodzongwa or musakama (Shona)	Stems, roots, bark fiber, ^a leaves, ^a fruits	Stomach pains, snakebites, headaches, diabetes, diuretic, laxative, antipyretic, insomnia, colic, constipation and venereal diseases, colds, cataracts, expectorant, bloody vomiting, bronchitis, diarrhea, dysentery, naso-pharyngeal affections; pulmonary troubles (Botermans et al., 2011; De Wet et al., 2012; Ruffo et al., 2002).	PMDN 492
<i>Tabernaemontana elegans</i> Stapf (Apocynaceae)	Toad tree (Eng.). Llaeveldse paddaboom (Afr.). UmKhahlwana, umKhadlu (Zulu). Muchanga (Shona)	Sap, leaves, roots, ^a rhizome ^a	Styptic, aphrodisiac, lung ailments, stomach ache, tuberculosis, venereal diseases, cancer (McGaw et al., 2008b; Neuwinger, 2000).	PMDN 466
<i>Warburgia salutaris</i> (Bertol.f.) Chiov. (Canellaceae)	Fever tree, pepper-bark, pepper-root tree, pepper-leaf tree, pepper-bark tree (Eng.). Koorsboom, peperbasboom, peperblaarboom, sterkbos (Afr.). Amazwechlabayo, isibaha, isibhaha (Zulu).	Stems, leaves and root bark ^a	Expectorant, colds, sinus clearing, spots in the lungs, malaria, sores in the mouth, skin irritations, rheumatism, pneumonia, purgative, headache, cough, constipation, aphrodisiac, abortion (Watt and Breyer-Brandwijk, 1962; Hutchings et al., 1996; Maroyi, 2013; Palgrave, 1977; Wyk and Gericke, 2000).	PMDN 634
<i>Trema orientalis</i> (L.) Blume (Cannabaceae)	Pigeonwood (Eng.). Hophout (Afr.). Ifamu, iphubane, isakasaka, isikhwelamfene, sakasaka, ubathini, umbengele, umbhangabhanga, umdindwa, umsekeseke, umvangazi (Zulu).	Fruit, stems, leaves, ^a bark, ^a twigs and seeds	Coughs, sore throats, asthma, bronchitis, gonorrhoea, yellow fever, toothache, antidote, dysentery, deworm, dogs (Rulangaranga, 1989).	PMDN 469
<i>Flueggea virosa</i> (Roxb.ex Willd.) Voigt (Euphorbiaceae)	Snowberry tree, white-berry bush (Eng.). Witbessiebos (Afr.). Isibangahlota sehlati, umyaweiyane (Zulu).	Roots, ^a fruits, barks, leaves	Snakebite, contraceptive, syphilis, diarrhea, rheumatism, sterility, rashes, malaria, respiratory tract infections and pneumonia (Ezeonwumelu et al., 2012).	PMDN 48
<i>Bauhinia petersiana</i> Bolle (Fabaceae)	Large white bauhinia (Eng.). Mubondo, mumwando, mun'ando or mupondo (Shona)	Roots, fresh leaves ^a	Dysmenorrhea female infertility, wounds, diarrhea, cough (Bosch, 2006).	PMDN 309
<i>Leucaena leucocephala</i> (Lam.) De Wit (Fabaceae)	Wild tamarind, white lead tree, lead tree, koa haole, ekoa, horse tamarind, jumbie bean, white popinac (Eng.)	Bark, leaves, seeds	Internal pain, contraceptive, ecobolic, depilatory, colds, fevers, flu, circulatory problems, to calm nerves, tuberculosis, reduce back pain and menstrual cramps, edible seed sufficiently cooked. (Pound and Martinez, 1983).	PMDN 343
<i>Heteropyxis natalensis</i> Harvey (Myrtaceae)	Lavender tree, natal lavender (Eng.). Lavender tree, laventelboom, (Afr.). Inkunzi (Zulu). Mudedede (Venda). Inkhuzwa (Zulu).	Leaves, roots, bark	Respiratory disorders, decongestant, anti-infectant (Wyk et al., 1997), tea (Wyk and Gericke, 2000), bleeding gums, bleeding disorders menorrhagia, aphrodisiac (Watt and Breyer-Brandwijk, 1962), decongestant, sedative, anticonvulsant, anti-infective, perfume and general tonic (Chagonda et al., 2000).	PMDN 354
<i>Rapanea melanophloeos</i> (L.) Mez (Myrsinaceae)	Cape beech, rapanea (Eng.). Boekenhout, Kaapse boekenhout, rooi-boekenhout, swartbas (Afr.). Ikhubalwane, inhluthe, isicalabi, isiqalaba-sehlati, umaphipha-khubalo, umhluti-wentaba, uvukwabafile (Zulu).	Grey bark, ^a roots, leaves	Respiratory problems, stomach, muscular and heart complaints (Wyk et al., 1997).	PMDN 330
<i>Rhamnus prinoides</i> L'Hérit. (Rhamnaceae)	Camdeboo, dogwood, glossy-leaf, shiny leaf, stinkwood (Eng.). Alinkbaar, hondepijsout, kamdeboo-stinkhout, seerkeelboom (Afr.). Ulenyeny, umgilindi, umhlinye, umnyeny (Zulu).	Fruit, leaves, bark, stems, twigs, seeds, roots ^a	Blood cleaning, pneumonia, rheumatism, sprains, stomach ache, gargle, skin complaints, respiratory infections, sexually transmitted disease, arthritis, cold, back pains, stomach ache, headache (Crowch and Okello, 2009).	PMDN 332
<i>Ziziphus mucronata</i> Willd. (Rhamnaceae)	Buffalo thorn, bogwood, cat-thorn (Eng.). Blinkbaar-wag'n-bietjie, buffelsdoring (Afr.). Isilahla, isulahlankosi, umlahlankosi, umlahlabantu, umkhobobonga, umphafa (Zulu).	Roots, barks, ^a leaves	Pains, dysentery, pulmonary ailments, septic swellings of the skin, boils, swollen glands, wounds and sores, snake bites, Coughs, chest ailments, (McGaw et al., 2008b; Hutchings et al., 1996).	PMDN 576
<i>Chaetacme aristata</i> Planch. (Ulmaceae)	Thorny-Elm, basterwitpeer (Eng.)	Leaves ^a	Tuberculosis, back wounds, spinal weakness (Newton-Fisher, 2006).	PMDN 588
<i>Clerodendrum glabrum</i> var. <i>glabrum</i> (Verbenaceae)	Tinderwood (Eng.). Moswaapeba (Sotho). Tontelhout (Afr.). Munukha-tshilongwe (Venda). Umqwaqwanam (Xhosa). Umqoqonga (Zulu). Xinhunwelambava (Tswana). Mohlokohlolo (Nsotho)	Roots, leaves, ^a barks	Snakebites, coughs, colds, prolapse, wounds and diarrhea, bad dreams, purgatives for calves, antiparasites, animal anthelmintics for dogs, calves and donkeys (Watt and Breyer-Brandwijk, 1962).	PMDN 728
<i>Lippia javanica</i> (Burm.f.) Spreng (Verbenaceae)	Fever tea, lemon bush (Eng.). Koorsbossie, beukesbossie, lemoenbossie (Afr.). Mutswane, umsutane (Swati). Inzinziniba (Xhosa). UmSuzwane, umSwazi (Zulu). Musukudu, bokhukhwane (Tswana).	Leaves, ^a twigs, ^a roots	Coughs, colds and bronchial problems, fever, asthma, chronic coughs and pleurisy, skin disorders (Hutchings et al., 1996.; Watt and Breyer-Brandwijk, 1962; Wyk et al., 1997).	PMDN 435

Eng. = English, Afr. = Afrikaans.

^a Used against tuberculosis or respiratory ailments.

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