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Full Length Research Paper

Antimicrobial activity of some medicinal plants used for the treatment of tuberculosis in the Eastern Cape Province, South Africa

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***Artemisia afra* Jacq. Ex Willd., *Carpobrotus edulis L.* and *Tulbaghia violacea Harv*. were screened for activity against *Bacillus cereus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Mycobacterium aurum* A+ strain using a two-fold microdilution bioassay. *M. aurum* is tuberculosis (TB) related strain that was used in this study. These plants were selected based on their use by South African traditional healers for the treatment of TB and symptoms of the disease. All three plants were extracted with water, ethanol and dichloromethane. The extracts of *A. afra* were found to be active against all the tested microorganisms. Only in the instance of *A. afra* and *C. edulis* did water extract show activity against *M. aurum* A+ strain. The ethanol extract of *C. edulis* showed very good activity against the Gram-positive bacteria only. Dichloromethane extracts of *T. violacea* were found to be highly active against all the microorganisms, except for *K. pneumoniae*.**

Key words: Medicinal plants, tuberculosis, antibacterial activity, *Mycobacterium aurum.*

INTRODUCTION

Tuberculosis (TB) remains one of the most prevalent causes of death in developing countries, due to a single infectious bacterial agent called *Mycobacterium tuberculosis* (WHO, 1998). Currently, one third of the world's population is infected with *M. tuberculosis* and each year there are 2 - 3 million deaths worldwide caused by the disease (Zumla et al., 1999). TB is a leading cause of death among people with human immunodeficiency virus (HIV). Individuals infected with HIV are very susceptible to TB and often develop this disease before other manifestations of AIDS become apparent (Grange and Davey, 1990; Lall and Meyer, 1999). Today, strains of TB that are resistant to all major anti-TB drugs have emerged. The emergence of resistance to antimicrobials, though is a natural biological occurrence, has become an important public health issue in many developing countries as the treatment of TB requires the use of more expensive drugs for a longer treatment period. There is, therefore, an urgent need for new, inexpensive TB drugs which are more effective and withless side effects. Medicinal plants are an integral part of South African culture. In South Africa, 21st century drug therapy is used alongside with traditional African medicines to heal the sick. While plants have been used in traditional medicine to treat various ailments, scientific analyses of the supposed benefits of many plants is still inadequate especially in southern Africa. *Artemisia afra* Jacq. Ex Willd., commonly known as 'Umhlonyane' in Xhosa, is a shrubby perennial herb with leafy and hairy stem. The plant is widely used for various medicinal purposes by different cultures in different geographical areas. *A. afra* has been traditionally used for respiratory disorders such as coughs, colds, whooping cough, bronchitis and asthma. It is also used in treating fevers, mumps swelling, pneumonia, pimples and skin rashes (Van Wyk and Gericke, 2000). *Carpobrotus edulis* L., also referred to as sour fig or Hottentot's fig, is widely utilized as a traditional remedy for a wide range of bacterial and fungal infections (Smith et al., 1998) including the treatment of eczema, burns, wounds, TB, vaginal thrush, toothache and earache (Van Wyk et al., 1997). *Tulbaghia violacea* Harv. is a small bulbous herb that grows in rocky grasslands. Its evergreen leaves exhibit a garlic-like smell and have been

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used in some cultures as a substitute for garlic and chive (Kubec et al., 2002). The plant is known by several common names including 'wild garlic' and 'society garlic'. *T. violacea* has been traditionally used for the treatment of colds, fever, asthma, TB, stomach problems and fits. It has also been reported that the Zulus grow *T. violacea* around their homes to repel snakes (Hutchings et al., 1996; van Wyk and Gericke, 2000).

Based on the ethnomedical information on these plants, they were screened against two Gram-positive bacteria, *B. cereus* and *S. aureus*, two Gram-negative bacteria, *Escherichia coli* and *Klebsiella pneumoniae*, and *M. aurum* A+ strain. *M. aurum* is a sister strain of *M. tuberculosis* which is the causative agent of TB. A study undertaken by Chung et al. (1995) has shown that the susceptibility of *M. aurum* closely resembled that of *M. tuberculosis* as compared to other strains of Mycobacteria. The other four bacteria were included because of their opportunistic properties. Immuno-compromised individuals are susceptible to a number of infections which are caused by a number of pathogens including the bacteria incorporated in this study. In this study, three traditionally used medicinal plants, viz., *A. afra, C. edulis* and *T. violacea* were investigated for their activity against five bacteria including *M. aurum*.

MATERIALS AND METHODS

Plant collection

The leaves of *A. afra* were collected from Alice, while *C. edulis* (leaves) and the bulb of *T. violacea* were collected from Grahamstown, in the Eastern Cape Province of South Africa (latitudes 30°00´- 34°15´S and longitudes 22°45´ - 30°15´E). A voucher specimen (Buwa 01, 02 and 03) for each plant was prepared and deposited in the herbarium of the University of Fort Hare. Each plant material was chopped into small pieces, air dried and ground to fine powder using a blender.

Plant extraction

Three separate samples of 1 g each were extracted with 10 ml water, 100% ethanol and dichloromethane respectively. Extraction was performed by shaking in these solvents for 24 h, at room temperature. The plant extracts were filtered through Whatman No. 1 filter paper and evaporated to small fractions under reduced pressure using a rotary evaporator. The filtrates were taken to total dryness in front of a fan until a constant dry weight of each extract was obtained. This was done by weighing plant extracts daily. The residues were stored at 10°C until used.

Bacterial strains and inoculum preparation

Bacillus cereus, *E. coli*, *K. pneumoniae,* and *Staphylococcus aureus* were laboratory isolates obtained from the Department of Biochemistry and Microbiology, University of Fort Hare, South Africa, while *M. aurum* A+ strain was obtained from the Microbiology Laboratory, Division of Pharmacology, University of Cape Town. *B. cereus*, *E. coli*, *K. pneumoniae* and *S. aureus* were maintained on nutrient agar plates and invigorated for bioassay by culturing a single colony in 2 ml nutrient broth for 24 h, after which the optical density (OD) at 600 nm for each liquid culture was determined. The saturated bacterial culture was then diluted with nutrient broth (1 ml bacteria: 99 ml broth), to make certain that the bacteria were at the start of the log phase when the test commenced.

Preparation of *M. aurum* A+ stocks

M. aurum A+ was maintained in Middlebrook 7H9 broth containing 10% OADC (oleic acid + albumin + dextrose + catalase). Inoculum was prepared by transferring the stock bacterial culture to supplemented 7H9 broth (Middlebrook 7H9 + 10% OADC) and grown for 72 h on a shaker. Two (5 ml) supplemented 7H9 broths were inoculated by the bacterial culture and grown for 72 h. Twenty percent sterile glycerol was added to each culture and 500 µl aliquots were made into sterile Eppendorf tubes. These stocks were named G1 stocks and were stored at -30°C. A single G1 stock was used to inoculate supplemented Middlebrook 7H10 agar (7H10 + 10% OADC) plates and incubated at 37°C for four days or until growth was observed. From this culture a single colony was used to inoculate 5 ml supplemented 7H9 broth. This was grown on a shaker at room temperature for 72 h and used for the experiment.

Microdilution bioassays

For the screening of the extracts against *B. cereus, E. coli, K. pneumoniae* and *S. aureus*, the microplate method of Eloff (1998) was used. Residues of plant extracts were redissolved with the extracting solvents at a concentration of 50 mg/ml. Dichloromethane extracts were redissolved in 25% ethanol. All extracts were initially tested at 12.5 mg/ml in 96-well microplates and serially diluted two-fold to 0.098 mg/ml, after which 100 µl bacterial culture were added to each well. Streptomycin (100 µg/ml) served as a positive control for each bacterium, whereas a solvent used to dissolve plant extracts was included as a negative control and bacteria free wells were included as blank controls. The microplates were covered and incubated for 24 h at 37°C. As an indicator of bacterial growth, 40 µl *p*-iodonitrotetrazolium violet (INT) (0.2 mg/ml) dissolved in water were added to the wells and incubated at 37°C for 30 min. MIC values were recorded as the lowest concentration of the extract that completely inhibited bacterial growth, that is a clear well. All extracts were tested in triplicates.

The broth microdilution method (Swenson et al., 1982) was used to determine the MIC of *M. aurum* A+ of the investigated plant extracts. The aqueous extract residues were redissolved in water and other extract residues were dissolved in dimethyl sulfoxide (DMSO). All extracts were dissolved to a concentration of 100 mg/ml. One hundred microliter of the supplemented 7H9 broth was added to all the wells of microtitre plates. All extracts were tested at a concentration of 25 mg/ml and serially diluted to 0.195 mg/ml. The optical density of the 72 h broth culture was determined and adjusted at 550 nm. One hundred microliter of the diluted culture was added to every well of the microtitre plate. The controls included the solvent used to dissolve plant extracts, Middlebrook 7H9 broth alone, and the antibiotic streptomycin (1.56 mg/ml) as a positive control. The plates were covered and incubated at 37°C for 72 h. After incubation, 40 µl of 0.4 mg/ml solution of INT was added to each well of the plate. The plates were covered and incubated for 24 h at 37°C. All extracts were tested in triplicates.

RESULTS

The leaves of *A. afra* showed the best MIC values for all

Table 1. Antibacterial activity tested in the microdilution bioassay of three plant species used in traditional medicine in South Africa.

Plant	Plant part	Solvent	Yield (mg)	MIC (mg/ml)				
				B. cereus	***E. coli***	***K. pneumoniae***	***S. aureus***	***M. aurum***
A. afra	Leaves	Water	1133.9	3.125	0.130	1.560	1.040	1.560
		Ethanol	759.3	0.163	0.130	0.780	0.098	3.125
		Dichloromethane	648	0.130	0.098	0.390	0.098	3.125
C. edulis	Leaves	Water	3351	1.040	6.250	6.250	6.250	4.690
		Ethanol	613.9	0.195	3.125	3.125	0.195	3.125
		Dichloromethane	900	1.560	1.560	1.040	1.300	6.250
T. violaceae	Bulb	Water	2554.8	4.166	3.125	8.330	2.600	Na
		Ethanol	249.3	3.125	1.560	3.125	1.560	3.125
		Dichloromethane	120	0.780	0.780	6.250	0.780	0.780
Streptomycin (µg/ml)				3.125	0.780	0.780	0.390	0.750

Na; not active at the highest concentration tested.

the tested extracts, including water (Table 1). The MIC values in aqueous extracts were high, especially against *E. coli*, a Gram-negative bacterium, followed by *K. pneumoniae* and *S. aureus*. It is worth noting that only in the case of *A. afra* and *C. edulis* did water extract show activity against *M. aurum* A+. This is interesting as drugs used by traditional healers are mostly prepared with water. The antimycobacterial activity for *C. edulis* was weak as compared to that of *A. afra*. *A. afra* has been reported for its use in traditional medicine to treat fever, colds, pneumonia and pulmonary TB. The ethanolic and dichloromethane extracts exhibited very good activity against all the tested microorganisms, with concentrations ranging from 0.163 to 0.098 mg/ml. Concerning *M. aurum* A+, the ethanolic and dichloromethane extracts had weak activity.

The ethanolic extract of *C. edulis* displayed strong activity against Gram-positive bacteria whereas weak activity was detected against *E. coli* and *K. pneumoniae* which are Gram-negative bacteria. The ethanolic extract displayed the lowest antimycobacterial activity as compared to both water and dichloromethane extracts.

T. violacea extracts showed the best MIC values for the dichloromethane extracts against all the tested bacteria excluding *K. pneumoniae*. The dichloromethane extract was exceptionally active against *M. aurum.* The aqueous extract of *T. violacea* displayed no activity against *M. aurum*.

DISCUSSION

TB is the leading killer of youths, women, and AIDS patients world wide (Mann et al., 2008). Although people with HIV/AIDS are dangerously susceptible to a number of opportunistic infections, TB is without doubt, the main cause of death. There is, therefore, the challenge to fight multidrug-resistant (MDR) TB. People infected with HIV and living with AIDS are at great risk for developing MDR TB. The search for new anti-tuberculosis drugs has become more important due to the above reasons and others such as shortage and expensive nature of TB drugs. Traditional medicine is a readily available alternative in the search for new antimycobacterial compounds. The plants screened in this study are used in traditional medicinal practices for the treatment of various diseases including TB.

The results obtained from this study justify the traditional use of *A. afra*, *C. edulis* and *T. violacea* for the treatment of TB or its symptoms. They also substantiate their potentials as sources of leads in the development of antituberculosis agents. However, the aqueous extracts of *C. edulis* and *T. violacea* displayed weak or no activity against *M. aurum*, even at concentration as high as 25 mg/ml. This may be due to the complex lipoglycan calyx on the cell surface which provides a significant physical barrier to intracellular acting compound (Ballell et al., 2005). Lack of penetration is thought to be a reason why many antibiotics show no activity against *M. tuberculosis* (Gao et al., 2003). Cell wall biosynthesis is believed to be a key target for antimycobacterial chemotherapy (Eldeen and van Staden, 2007).

Although water is the most commonly solvent used by traditional healers to extract the active compounds due to its availability, antimycobacterial screening of *T. violacea* generally resulted in higher inhibitory activity from dichloromethane extract as compared to water and ethanol. This suggests that water is not the most effective solvent at extracting the active compounds from the plant. However, considering the fact that the dosage prescribed by the traditional healers is usually very high, for instance three to four cupfuls per day for adult, water can still be considered as an appropriate extracting solvent for traditional remedies (Shale et al., 1999). Conversely, negative results do not mean absence of bioactive constituents nor that the plant is inactive, plant extracts

may act in other ways by stimulating the immune system of the patient, or by creating internal conditions that are unfavourable for the multiplication of the microorganism. Newton et al. (2002) reported that there were a number of plants, supposedly used in traditional medicine to treat TB, which did not demonstrate any antimycobacterial activity against *M. aurum* and *M. smegmatis*. The author suggested that the plants may be used to treat the symptoms of the disease rather than actually cure the disease itself. Although these plants are traditionally used for treating TB and other respiratory diseases, it is possible that these plant extracts are effective against ailments such as bronchitis, cough and asthma caused by agents other than *Mycobacteria*. Screening directly against TB agent is unsafe because of the highly infectious nature of the pathogen and is time consuming as the organism is relatively slow growing (Bloom, 1994).

In the current study, the extracts of *A. afra* leaves showed the best activities. A number of studies have revealed that the essential oil of leaves of *A. afra* contain 1,8-cineole, α-thujone, β-thujone, camphor and borneol, which are known to exhibit antimicrobial properties (Graven et al., 1992; van Wyk and Gericke, 2000). The oil may be responsible for some of the activities of this plant. 1.8-cineole has been reported to exhibit significant antimicrobial activity against certain Gram-negative and Gram-positive bacteria including *E. coli*, *B. subtilis*, *S. aureus* and *Mycobacterium phlei* (Pooter *et al*., 1995). Aqueous extracts of *A. afra* also contain flavonoids which have been reported to possess antimicrobial, anti-inflammatory, anti-oxidant and anti-mutagenic properties (Rice-Evans, 1997; Tang et al., 2000; van Wyk and Gericke, 2000; Miura et al., 2003; Sadik et al., 2003; Remberg et al., 2004). In a study done by McGaw et al. (2000), the ethanolic extract of *A. afra* exhibited some activity whereas *T. violacea* had no activity.Very good activity was also observed with ethanol extract of *C. edulis* leaves against *B. cereus*, *S. aureus* and *M. aurum*. In a study undertaken by Van der Watt and Pretorius (2001) five active flavonoids isolated from *C. edulis* were purified and identified as rutin, neohesperidin, hyperoside, cactichin and ferulic acid. These flavonoids showed activity against *B. subtilis*, *S. aureus*, *S. epidermidis*, *Streptococcus pneumoniae*, *Moraxella catarrhalis* and *Pseudomonas aeruginosa*.

A study undertaken by Martins et al. (2005) have shown that the methanol extract of *C. edulis*, inactive against the methicillin-resistant *S. aureus* or the multi-drug-resistant *M. tuberculosis*, does inhibit the growth of these two bacteria once they are phagocytosed by monocyte derived human macrophages. Our results correlate with the antimicrobial effects observed with the traditional uses of *C. edulis* reported by Van Wyk et al. (1997) and Martins et al. (2005) and suggest that this plant may serve as a source of new antimicrobial agents that are effective against problematic drug-resistant infections., *C. edulis*, *C. edulis* and *T. violacea* that are used against TB in the Eastern Cape Province of South Africa, exhibited significant antibacterial activities which may explain and justify the usage of these plants by traditional healers. It has also highlighted the importance of assessing the herbal remedies used by traditional practitioners. It has shown that some plant extracts, particularly *T. violaceae* water extract, may not contain compounds which inhibit the growth of or kill *M. aurum* but it is possible that the may have stimulant effects on the immune system. However, no compounds from extracts of these plant species with antimycobacterial activity have been reported so far. Further investigation on seasonal variation study and bioassay-guided isolation of these plants is being done.

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