

Full Length Research Paper

Antibacterial effectiveness of *Tetradenia riparia* extract, a plant traditionally used in the Eastern Cape Province to treat diseases of the respiratory system

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The antibacterial properties of *Tetradenia riparia*, the most frequently used plant by the traditional healer for the treatment of chest and cough related infections was reported in this study. The plant was investigated to evaluate claims made by the users as a remedy for chest and cough related infections. Ten bacterial strains (five gram-positive and five gram-negative) were used for the antibacterial assays. All the extracts showed some activity against the bacteria tested at concentrations ranging from 1.0 to 10.0 mg/ml, with the exception of dichloromethane extract which did not inhibit any of the microorganisms used. The antibacterial properties of the plant extracts were more visible with the gram-positive bacteria while gram-negative bacteria showed more resistance to the treatments especially at low concentration. The resistance of gram-negative bacteria has been attributed to the composition of their cell walls.

Key words: *Tetradenia riparia*, medicinal plants, antibacterial activity, bacterial strain.

INTRODUCTION

The continuous use of synthetic drugs such as antibiotics for so many years has led to the resistance of bacterial strains, thus there is a great demand for development of new forms of drugs that are more effective against bacteria (World Health Organisation, 2004). Recently, real time evolution of resistance in *Escherichia coli* populations was demonstrated with a device that allows for continuous culture of bacteria under a constant drug

selection pressure using computer feedback control of antibiotic concentration (Rosenthal and Elowitz, 2012). Thus, mutative evolutionary development of bacterial resistance has been demonstrated in real time.

The spread of diseases such as tuberculosis caused by *Mycobacterium tuberculosis* as well as other opportunistic *Mycobacterium* infections has grown rapidly most especially in countries that do not have well

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Table 1. Summary of extraction yields from the dry leaves of Leaves of *T. riparia*.

Extraction solvent	Volume (L)	Amount of material (g)	Yield (g)	Yield (%)
Ethyl acetate			14.90	2.13
Dichloromethane			14.30	2.04
Hexane	1.5	700	15.17	2.17
Methanol			09.70	1.39
Water			25.00	3.57

established health care systems and facilities, making the lengthy expensive treatments a challenge (Zager and McNerney, 2008). The discovery of drug resistant strains of *M. tuberculosis* such as multiple drug resistant (MDR) and extremely drug resistant (XDR) strains has made the situation worse (Jones et al., 2008). Even though the number of tuberculosis (TB) related deaths globally look stable for now at about 2 million per annum, new infections are rising mostly due to the human immunodeficiency virus (HIV) epidemic (Gutierrez-Lugo and Bewley, 2008).

Traditional medicine has remained the most affordable and easily accessible source of treatment in the primary healthcare systems of resource poor communities where the local people have a long history of traditional plant usage for medicinal purposes (Maroyi, 2013). Traditional medical practitioners in developing countries are known to use plants as forms of drugs to heal many kinds of diseases. However, most plants used in this way by traditional healers have not been scientifically tested. Most new drugs come from natural products such as plants (Newman and Cragg, 2007). Plants are known to be important sources of highly active anti-mycobacterial agents (Gibbons, 2005; Pauli et al., 2005). South Africa prides itself with a large floral diversity and a long historical usage of medicinal plants by traditional healers and knowledgeable elders. In the Eastern Cape Province of South Africa, studies have focused on the treatment of diabetes (Erasto et al., 2005), HIV and acquired immune deficiency syndrome (AIDS) (Omoruyi et al., 2012) and opportunistic fungal infections (Otang et al., 2012).

Coopoosamy and Naidoo (2011) assessed the potential of *Tetradenia riparia* in the treatment of common skin conditions in some rural communities of South Africa and found the plant to be quite effective. Herbal plants play a fundamental role in the treatment of coughing and chest related diseases particularly in remote areas of the Eastern Cape where health care facilities are sparsely located. In a parallel study, the plants implicated in the treatment of coughing and chest related diseases in the OR Tambo District Municipality were documented. In this study, which was also aimed at determining the most commonly used plants and plant parts, the methods of preparation and the methods of application, *T. riparia*

plant was mentioned as the most frequently used plant by the traditional healer for the treatment of chest and cough related infections. This study aims to validate this claim by testing the antibacterial effectiveness of *T. riparia* plant extract on ten different micro-organisms, which are notorious for causing coughing and chest related diseases.

MATERIALS AND METHODS

Hexane, ethyl acetate, methanol and dichloromethane were obtained from Sigma-Aldrich as analytical reagents. The orbital shaker used was an MRC with twelve positions for 250 to 500 ml Erlenmeyer flasks. The Büchner funnel used for filtration was a Corning and the filter paper was a Whatman No. 1. The rotary evaporator was a Buchi R-215 fitted with a vertical water cooled condenser. Nutrient agar was purchased from Arcos. The autoclave was an Optima B class from Prestige Medical. The water was triple distilled and passed through a de-ionising column before use. The ten bacterial strains were obtained from the National Health Laboratory Services. All nutrient media were from Acros (typically 0.4 g L⁻¹, KH₂PO₄, 0.05 g L⁻¹ MgSO₄ × 7H₂O, 0.1 g L⁻¹ NaCl, 0.5 g L⁻¹ NH₄H₂PO₄, 0.01 g L⁻¹, FeCl₃, 3 g L⁻¹ yeast extract, 1 g L⁻¹ glucose 15 g L⁻¹, agar). *T. riparia* plant was collected from the wild in the Eastern Cape Province of South Africa. This plant is known as Iboza by the Xhosa people residing in the study area (Coopoosamy and Naidoo, 2011). The plant was identified at the Kei Herbarium of Walter Sisulu University (yndamane 4953).

Extract preparation

The leaves of *T. riparia* were air-dried avoiding direct exposure to sunlight and then ground into fine powder using a pastel and mortar. Portions of this plant material (700 g) were each shaken with 1.5 L of ethyl acetate, dichloromethane, hexane, methanol and water with an orbital shaker for 24 h. Filtering of extracts was done with a Büchner funnel on Whatman No. 1 filter paper under suction. The extracts were concentrated to dryness with a rotary evaporator under reduced pressure at 30 to 40°C. The extraction yields are summarized in Table 1. Stock solutions were prepared by re-suspending 3 g of each of the extracts in 50 mg/ml of the solvent used for the extraction (Taylor et al., 1996; Koduru et al., 2006).

Antibacterial screening

Hexane, ethyl acetate, methanol, dichloromethane and aqueous extracts of *T. riparia* leaves were screened against ten bacterial

Table 2. Antibacterial activities of *T. riparia* extracts against test organisms.

Bacterial species	MIC (mg/ml)					
	Gram +/-	Ethyl acetate	Dichloromethane	Hexane	Methanol	Water
<i>Staphylococcus aureus</i>	+	5.0	Na	10.0	1.0	5.0
<i>Staphylococcus epidermidis</i>	+	1.0	Na	10.0	10.0	5.0
<i>Bacillus cereus</i>	+	1.0	Na	10.0	1.0	5.0
<i>Micrococcus kristinae</i>	+	1.0	Na	10.0	5.0	Na
<i>Streptococcus faecalis</i>	+	5.0	Na	10.0	10.0	5.0
<i>Escherichia coli</i>	-	10.0	Na	10.0	10.0	10.0
<i>Pseudomonas aeruginosa</i>	-	10.0	Na	10.0	10.0	Na
<i>Shigella flexneri</i>	-	10.0	Na	10.0	10.0	Na
<i>Klebsiella pneumonia</i>	-	10.0	Na	10.0	10.0	10.0
<i>Serratia marcescens</i>	-	10.0	Na	10.0	10.0	10.0

MIC = Minimum inhibitory concentration, Na = not active.

species, using the agar-diffusion method.

Preparation of agar-extract plates

Nutrient agar was prepared and then autoclaved before it was allowed to cool to about 60°C then the extracts were added. The agar medium containing extracts at concentrations of 1.0, 5.0, 10.0 mg/ml were poured into petri dishes and then swirled carefully until the agar began to set and were left overnight in order for the solvent to evaporate (Afolayan and Meyer, 1997; Grierson and Afolayan, 1999a). Blank plates were used as control containing nutrient agar with respective solvents of extraction only, but without any extracts.

Antibacterial testing

Ten bacterial strains obtained from National Health Laboratory Services (NHLS) were used for this study as shown in Table 2. Each organism was maintained on a nutrient agar plate and was recovered for testing by growth in nutrient broth for 24 h before testing. All the cultures were diluted 1:100 with fresh sterile nutrient broth. Organisms were then streaked in a radial pattern on agar plates and incubated at 37°C for 24 to 48 h (Afolayan and Meyer, 1997; Grierson and Afolayan, 1999b; Aliero et al., 2006). In order for the extract to be declared active, complete suppression of growth was required.

RESULTS

Table 1 summarizes the results of extraction from the dry leaves of *T. riparia* with the extraction amounts in grams and percentage yields. The table also shows that all extractions were performed using 1.5 L of extraction solvent and 700 g of ground dry leaves of *T. riparia*. The antibacterial activities of *T. riparia* extracts against test organisms are tabulated in Table 2 and illustrated in

Figure 1. Table 2 shows the minimum inhibitory concentrations (MIC) calibrated into 1.0, 5.0 and 10.0 and na (not active), where 1.0 represents the highest activity, 10.0 the lowest activity and na means the extract was not active at any of the concentration used. Figure 1 is a bar graph showing the activity of the extracts against the bacterial strains. The dichloromethane extract is not shown in this bar graph because it was not active against any of the organisms.

DISCUSSION

Out of a total of 17 plant species that were reported for the treatment of chest and coughing related diseases, *T. riparia* was the most frequently cited. Therefore, *T. riparia* was extracted as already outlined. Upon screening of the five extracts, the dichloromethane extract was the only extract that did not show any activity against bacterial strains. The other four extracts exhibited various degrees of activity with the ethyl acetate extract showing the highest activity against the bacterial species used during the antibacterial assays. The ethyl acetate extract was active against all bacterial species tested, with MIC values ranging from 1.0 to 10.0 mg/ml. Most of the bacteria were inhibited at 1.0 and 5.0 mg/ml. The dichloromethane extract was not active against any of the bacterial species, demonstrating its inability to extract active principles that could be used to inhibit growth of bacteria.

The hexane extract was effective against all the bacterial strains at a concentration of 10.0 mg/ml. The aqueous extract was active against most of the bacteria with MIC values ranging between 5.0 and 10.0 mg/ml. Results from this extract may justify the use of *T. riparia*

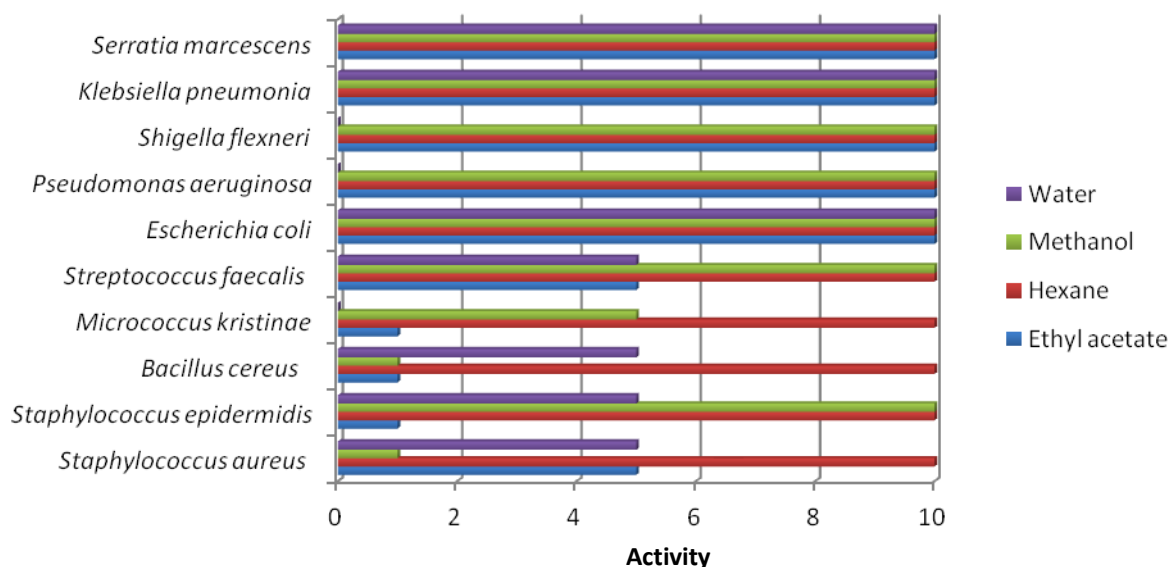


Figure 1. Antibacterial activities of *T. riparia* extracts against ten test organisms.

by herbalists as a remedy for persistent coughing. The methanol extract was active against all bacterial species, with MIC values ranging from 1.0 to 10.0 mg/ml. Moreover, most microbes were inhibited at the maximum concentration of 10.0 mg/ml, with two bacterial strains *Staphylococcus aureus* and *Bacillus cereus* (a virulent human pathogen) inhibited at the minimum concentration of 1.0 mg/ml.

The findings of the studies of Eloff (1998), Karaman et al (2003) and Steenkamp et al. (2004), that methanol extracts were more active than water extracts is in full agreement with the results of this study. The methanol extract was active against all bacterial strains used, while the water extract was not active against *Micrococcus kristinae*, *Pseudomonas aeruginosa* and *Shigella flexneri*. Interestingly, in an *in vitro* screening for antibacterial activity of hexane, ethanol and water extracts of South African plants conducted by the South African Medical Research Council (SA-MRC), no inhibitory activity of *T. riparia* against *S. aureus*, *B. subtilis*, *E. coli* or *Klebsiella pneumoniae* was demonstrated (McGaw et al., 2000). It is not clear why no inhibitory activity was demonstrated, since McGaw et al. (2008) also used the disc-diffusion and the micro dilution assays.

Ethyl acetate, hexane and methanol extracts inhibited all the bacteria tested, and the extracts showed consistence in inhibiting all the gram negative bacteria at 10 mg/ml. The inactiveness of the dichloromethane extract is noteworthy; as the study show that dichloromethane did not extract any of the active ingredients from *T. riparia* under the extraction procedure

used; however this is in contrast with Leitão et al. (2012) report who isolated a number of bioactive diterpenes from the dichloromethane extracts of *T. riparia* leaves, using high-speed counter-current chromatography (HSCCC). In addition, other researchers have reported extractions with a mixture of dichloromethane and methanol (York et al., 2012; Sajida, 2012).

The ethyl acetate extract inhibited both gram-negative and gram-positive bacteria and showed appreciable antimicrobial activity against gram positive bacteria with MIC ranging between 1.0 and 5.0 mg/ml. The methanol extract also showed appreciable inhibitory effects against gram-positive bacteria as most of them were inhibited at the low concentrations of 1.0 and 5.0 mg/ml followed by water extract with MIC values of 5.0 mg/ml against gram positive bacteria, and the hexane extract with MIC of 10 mg/ml. The activity of the extracts against *B. cereus* is noteworthy because it is a human pathogen that is difficult to treat with conventional antibiotics (Sidambiwe et al., 1999; Mathekgga et al., 2000).

S. aureus bacterial species are under the category of the hardest spore forming bacteria; they can thrive in any physiological environmental conditions. They can be cultured in an array of media ranging from dry to wet clinical materials for a very long period of time, they possess an element of heat resistance and are tolerant to high salt media (Islam et al., 2008). Therefore it does not come as a surprise that despite the presence of potent antimicrobial agents and improved public health conditions together with hospital infection control measures in place, they continue to be a human

pathogen of major concern. Unequivocally, the new antibiotics that have been developed together with increasing drug resistance and prevailing epidemiologic conditions have refurbished this microbe to be a pathogen of great concern for human diseases (Gerald et al., 2000). Nevertheless the ethyl acetate extract, hexane extract, methanol extract and water extract of *T. riparia* were effective against *S. aureus* with appreciable results especially the methanol extract with an MIC of 1.0 mg/ml.

The antibacterial properties of the plant extracts were more evident with the gram-positive bacteria while gram-negative bacteria showed resistance to the treatments especially at low concentration. Similar observations were reported by other workers (Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995). The resistance of gram-negative bacteria has been attributed to its cell wall, which is a complex unit consisting of an outer membrane and underneath, a peptidoglycan which adheres to the cytoplasmic membrane. Its cell wall membranes are capable of modifying enzymes, making them harmless to the bacteria (Boyd, 1988).

Conclusion

We have tested the extracts of *T. riparia*, one of the traditional plants used in the Eastern Cape province of South Africa to treat diseases of the respiratory system, for anti-bacterial activity against ten bacterial strains. This was a preliminary study for this plant to scientifically validate its traditional use. The ethyl acetate and methanol extracts of this plant showed effective antibacterial activity. However the dichloromethane extracts showed no activity. It is concluded therefore that the study provides a scientific basis for the traditional prescription and use of this plant to treat diseases not only of the respiratory system, but also other diseases that are caused by infection with the bacterial strains investigated. It is also postulated that the reason why the dichloromethane extract showed no antibacterial activity against the ten bacterial strains is probably that dichloromethane did not extract any of the active compounds that were extracted by the other solvents. This postulate will be investigated further using bioautography and thin layer chromatography. Further work to isolate and identify the chemical compounds that are responsible for the observed antibacterial activity is currently underway.

Furthermore, the antibacterial properties of *T. riparia* that have been observed in this study justify its use for treating chest and cough related disease, and work is in progress to isolate bioactive principles that may be responsible for the inhibitory effects recorded in this study. Some of the compounds that have been isolated from *T. riparia* include diterpenes such as ibozol (Zelnik

et al., 1978), 7- α -hydroxyroyleanone, 8(14),15-sandaracopimaradiene-7- α ,18-diol (Van Puyvelde et al., 1987), α -pyrones such as umuravumbolide (Van Puyvelde et al., 1979; Davies-Coleman and Rivett, 1995), tetradenolide (Van Puyvelde and de Kimpé, 1998), α -terpineol, fenchone, β -fenchyl alcohol, β -caryophyllene, perillyl alcohol and phytosterols (Campbell et al., 1997).

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