

Screening of *Commiphora schimperi* (O. Berg) from Iramba district in Tanzania for antibacterial activities

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SUMMARY

Commiphora schimperi gum-resin from Iramba district, Tanzania was screened for *in vitro* antibacterial activities by disk diffusion method. The bacteria employed were Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Salmonella gallinarum* and *Escherichia coli*). The results indicated that *C. schimperi* methanol and petroleum ether extracts were effective against *S. aureus* and *B. subtilis*. The methanol extract (200 mg/mL) caused complete zone of inhibition (17.6 ± 0.5 mm) against *S. aureus* while petroleum ether extract (200 mg/mL) caused a mean inhibition zone of 12.3 ± 0.9 mm against *B. subtilis*. This preliminary finding shows that *C. schimperi* gum-resin has antibacterial effects on some Gram positive bacteria and supports at least in part, the traditional knowledge of local users. Thus, *C. schimperi* gum-resin can be further subjected to phytochemical, pharmacological and toxicological evaluation.

Keywords: antibacterial activity, *Commiphora schimperi*, crude extract

INTRODUCTION

The Genus *Commiphora* includes 150–200 species widespread in the drier parts of tropical Africa particularly in Eastern Africa, with few species also occurring in Arabia and India. In Tanzania, the *Commiphora* spp. is mostly found in semiarid areas particularly in central regions (Singida and Dodoma) and in northern regions (Arusha and Kilimanjaro) (Hines and Eckman, 1993; Minja, 1999). Different species of *Commiphora* are used as sources of allopathic medicines, food, animal feeds and other general purpose uses (Paraskeva *et al.*, 2007; Tadesse *et al.*, 2007). In Tanzania for example, *Commiphora* is used as fodder for camels and goats and it is believed to possess antimicrobial and other medicinal properties (Hines and Eckman, 1993; Seed leaflet, 2008). Resin of *Commiphora* spp.

is the most important masticant among the Maasai and Batemi communities. The *C. schimperi* shrub extracts are also used in control of ectoparasites in animals, treatment of abscesses, dysentery, gastrointestinal ulcers, ringworm, wounds, rheumatism and helminthosis (Desta, 1995; Ibrahim and Ibrahim, 1998; Kaoneka *et al.*, 2007). In Tanzanian local languages, the *Commiphora* is known by different names like *Mturituri* (Swahili), *Oltেমwai* (Maasai), *Mguta* (Sukuma), *Dumbechanda* (Taturu) and *Mzilanzi* (Gogo) (Minja 1999; Sambuta and Masola, 2008).

In Tanzania like in any other developing countries where medical and veterinary facilities cannot satisfy national demands, traditional medicine plays a big role in combating both human and animal diseases through the use of traditional healers (Maregesi *et al.*, 2008). The importance of

using medicinal plants can be attributed to a number of reasons, including affordability and limited availability of western medicine as well as the trust in herbal medicine as an outcome from the witnessed positive results when applying herbs (WHO, 2002). More than 20,000 plant species are reported by WHO to be used for medicinal purposes (Gullece *et al.*, 2006). However, most of medicinal plants have not been scientifically tested or documented (Swaleh, 1999; Maregesi *et al.*, 2008). Despite the extensive traditional use of *C. schimperi* for treatment of different ailments in central Tanzania (Emmanuel Macha, Personal observation, 2009), the plant has not been pharmacologically tested. Claims of the efficacy of *C. schimperi* in its traditional usage therefore require validation and accurate documentation. Thus, this study aimed to assess the antibacterial effects of *C. schimperi* crude extracts against selected Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram negative (*Salmonella gallinarum* and *Escherichia coli*) bacteria.

MATERIALS AND METHODS

Crude extracts (gum resin) collection

The choice of the *C. schimperi* shrub to be tested for antibacterial activities based on the ethnopharmacological use through interviews with traditional healers. The crude extract (gum resin) was collected in February, 2009 in Mitala village, located in Iramba district of Singida region. By using a machete, stem incisions was made to enhance oozing of the gum resin. The gum resin was collected from different trees and pooled into sterile glass bottles and stored in cool box with ice pack during field sampling. The samples were subsequently transported to the laboratory at the Faculty of Veterinary Medicine, Sokoine University of Agriculture, for antibacterial

analysis. Identification of collected *C. schimperi* shrubs was done at the Department of Forest Biology, Sokoine University of Agriculture by Professor R.P.C. Temu. Voucher specimens were deposited in the same herbarium.

Preparation of the extract

Extraction of C. schimperi gum-resin with methanol

The extraction of *C. schimperi* gum-resin was done as described by Salamah and Zaid (1999) with some modifications. The extracts were all made with analytical grade solvents (Merck). One hundred grams of *C. schimperi* gum-resin was added with 200 mL of absolute methanol, thoroughly shaken to mix and left overnight at room temperature (25 °C). The mixture was separated by centrifugation (at 8000 rpm for 10 min) then the supernatant was decanted as methanol extract. This was repeated twice and the methanol extract was pooled in one bottle. The extract was concentrated in a rotary evaporator and further dried in a desiccator for three days. The extract was re-dissolved in the methanol to give a concentration of 200 mg/mL which was used in the antibacterial assay.

Extraction of C. schimperi gum-resin with other solvents

Two hundred grams of *C. schimperi* gum-resin was subjected to successive extraction with different solvents according to their polarities. The solvents used were petroleum ether, dichloromethane and ethanol, respectively. The 200 g of *C. schimperi* gum-resin was first mixed with 200 mL of absolute petroleum ether, mixed thoroughly and left overnight at room temperature (25 °C) to extract. The mixture was separated by centrifugation (at 8000 rpm for 10 minutes) and the supernatant was decanted as petroleum ether extract. The extraction was repeated for

dichloromethane and ethanol solvents. This resulted in three extracts namely: (i) petroleum ether extract; (ii) dichloromethane extract; and (iii) ethanol extract. Each extract was concentrated in a rotary evaporator, further dried in a desiccator for three days to allow more evaporation of the remaining solvents. Each solid extract was re-dissolved in the methanol to give a concentration of 200 mg/mL which was used in the antibacterial assay.

Antibacterial susceptibility testing

Antibacterial susceptibility test was performed on Muller Hinton (MH) Agar (Oxoid Ltd, Basingstoke, UK) by agar disc diffusion method as described by Luangtongkum *et al.* (2007) with some modifications. *B. subtilis* and *S. aureus* cultures were used as Gram positive bacteria while *E. coli* and *S. gallinarum* as Gram negative bacteria. All test bacteria were obtained from the research laboratory of the Department of Veterinary Medicine and Public Health, Sokoine University of Agriculture, Morogoro, Tanzania. Briefly, the MH agar was prepared in sterile glass plates. Each of the bacteria suspensions was prepared in a sterile normal saline and the suspensions were adjusted to a turbidity equivalent to a 0.5 McFarland standard. Sterile cotton-tipped swabs were used to transfer the inocula onto Mueller-Hinton plates to produce a confluent lawn of bacterial growth.

Then 100 µl of the methanol and petroleum ether extracts was spotted onto paper discs (6 mm) and dried under sterilized conditions. Another set of paper discs were spotted with dimethylsulfoxide (DMSO) and were used as negative controls. In each test performed, streptomycin (10 µg), amoxicillin (10 µg) and gentamicin (10 µg) (Span Diagnostic, Surat India) antibiotic discs were used as positive control.

After the inocula on the plates were dried, the test discs were distributed over the inoculated plates by using a sterile forceps. Each plate was added with one disc of methanol extract (test material); streptomycin (10 µg), amoxicillin (10 µg) and gentamicin (10 µg) discs (positive controls) and DMSO as a negative control. The same was done for the petroleum ether extract. Each bacteria species was tested in triplicate for each extract. The plates were incubated at 37 °C for 24 h. After the incubation period, the plate cultures were examined for inhibition zones around the wells. Results were recorded as presence or absence of zone of inhibition (Lennette, 1995) and the diameters of inhibition zones were measured with slipping callipers. The inhibitory zone around test paper disks indicated absence of bacterial growth and it was reported as positive (growth inhibition observed) and absence of zone as negative. For test and interpretation of the results the general guidelines of NCCLS (2002) and Gaudreau and Gibert (1997) were followed.

RESULTS

The results of the current study revealed that *C. schimperi* gum-resin has antibacterial effects on some Gram positive bacteria, in particular *S. aureus* and *B. subtilis* (Table 1). None of the extracts showed any activity against the Gram-negative bacteria, *S. gallinarum* and *E. coli*. The *C. schimperi* methanol extract (200 mg/mL) caused complete zone of inhibition (17.6 ± 0.5 mm) against *S. aureus* which is higher than the inhibition zone caused by 10 µg of amoxicillin and 10 µg of gentamicin. *C. schimperi* petroleum ether extract (200 mg/mL) caused a mean inhibition zone of 12.3 ± 0.9 mm against *B. subtilis* which is higher than the inhibition zone caused by 10 µg of amoxicillin on the same bacteria (Table 1).

Table 1. Antibacterial effectiveness of methanol, petroleum ether extracts and standard antibiotic discs

Bacteria	Mean inhibition zone \pm Std deviation (mm)					
	Methanol extract (50%)	Petroleum ether extract (50%)	Streptomycin (10 μ g)	Amoxicillin (10 μ g)	Gentamicin (10 μ g)	DMSO
<i>S. gallinarum</i>	Niz	niz	12.1 \pm 0.3	18.0 \pm 0	17.3 \pm 0.1	niz
<i>E. coli</i>	Niz	niz	14.3 \pm 0.6	14.3 \pm 0.7	15.0 \pm 1.1	niz
<i>S. aureus</i>	17.6 \pm 0.5	9.3 \pm 0.4	niz	10.0 \pm 0	15.2 \pm 0.6	niz
<i>B. subtilis</i>	11.2 \pm 1.1	12.3 \pm 0.9	20.4 \pm 0.2	11.9 \pm 0.6	17.0 \pm 0	niz

Legend: niz: No inhibition zone

DISCUSSION

The purpose of this study was to assess the antibacterial effects of *C. schimperi* crude extracts against selected Gram positive (*S. aureus* and *B. subtilis*) and Gram negative (*S. gallinarum* and *E. coli*) bacteria. It was generally found that *C. schimperi* gum-resin has antibacterial effects on some Gram positive bacteria, in particular *S. aureus* and *B. subtilis*. This gives some preliminary evidences on the traditional use of *C. schimperi* in medical and veterinary practices in Tanzania. A study by Paraskeva *et al.* (2007) on crude extracts from 10 South African *Commiphora* species also exhibited high antibacterial activity mostly against the Gram-positive bacteria.

However, none of *C. schimperi* crude extracts showed antimicrobial activities against Gram negative bacteria. The lack of activity of tested *C. schimperi* gum-resin may be explained by the fact that unlike Gram-positive bacteria, the lipopolysaccharide layer along with proteins and phospholipids are the major components in the outer surface of Gram-negative bacteria which offer a much more complex barrier system against permeation of foreign substances (in this case, the antibacterial agent) (Palombo and Semple, 2001; Denyer and Maillard, 2002). This

also may apply on the different methods of preparing extracts from that used by traditional healers which may have led to separation or loss of some active ingredients. The traditional healers use the whole plant part without extraction or fractionation. In this way, a number of compounds when are acting synergistically may produce a particular therapeutic effect which may be missed upon separation. Further causes of these differences in antibacterial activity may well be found in the inherent differences in strain sensitivity, and the mode of and choice of solvent for extraction.

The selection of *C. schimperi* for this study was based on its use to treat infectious diseases such as abscesses, dysentery, diarrhoea, ulcers and wounds. *S. aureus* was used since is amongst the microbes responsible for causing abscesses and sepsis of wounds, which were indicated to be treated by several plants by traditional healers. Nevertheless, *B. subtilis* and *E. coli* have been known to act as primary invaders or secondary infectious agent in a number of diseases and have been implicated in some cases of food poisoning and cause of dysentery (Turnbull and Kramer, 1991). *S. gallinarum* is known in causing diarrhoea and other signs in chickens. *C. schimperi* as an herbal medicine of the indigenous people in

central and northern Tanzania may have helped to combat these microbes.

The antibacterial activity was also observed to differ based on the extracting solvent. The *C. schimperi* methanol extract was effective against *S. aureus* where it resulted into a complete zone of inhibition of 17.6 ± 0.5 mm at a concentration of 200 mg/mL which was comparable to that of gentamicin (10 µg) (Table 1). Similarly, petroleum ether extract caused a mean inhibition zone of 12.3 ± 0.9 mm comparable to that of amoxicillin (10 µg). The high activities revealed by the methanol and petroleum ether extracts may be due to high polarity of these solvents which naturally has the ability of extracting high quantity of phytoconstituents (Marjorie 1999). Our results are also in agreement with those of Salamah and Zaid (1999) and Paraskeva *et al.* (2007) who observed differences in antibacterial activities of *Commiphora* spp. that varied depending on the extracting solvents. In addition, the high antibacterial activities in methanol extract may be ascribed to the presence of polyphenol compounds such as tannins which are known to have a wide range of non-specific anti-infective actions. However, if tannins were solely responsible for the activity presented by these results, this activity would be observed against all organisms and would not be limited to Gram-positive bacteria. Thus, tannins may be partially responsible for the antibiotic activity observed in Gram-positive bacteria.

Based on polarity of the extracting solvents, petroleum ether extracts ranked highest (42%) followed by methanol extracts (24%) then dichloromethane (2.4%) and least active were ethanol extracts (1.9%). The results clearly indicate that the total extractable lipids of resin-gum varied according to the solvent used. Our results are in line with the findings by

George and Pandalai (1949) who reported that among organic solvents, petroleum ether was the best extracting solvent for medicinal plants. It was also noted that, successive extraction and concentration using rotary evaporator was a good method over direct methanol extraction of *C. schimperi* gum-resin.

It is therefore concluded that although some studies reported on exotic *C. schimperi*, this study represents the first account on the *in vitro* antibacterial potential of this natural product from Iramba district, Tanzania. The *in vitro* investigations indicated that *C. schimperi* gum-resin display promising antibacterial activity against Gram positive bacterial species, in particular *S. aureus* and *B. subtilis* hence this plant can be further subjected to phytochemical, pharmacological and toxicological evaluation. This observation provides a scientific basis for use of this plant in traditional medicine, especially in the treatment of wounds and diarrhoea where *S. aureus* and *B. subtilis* are commonly involved.

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