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Full Length Research Paper

Effect of crude extracts from *Commiphora swynnertonii* (Burtt) against selected microbes of animal health importance

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Ethanolic extracts from resin, root bark, stem bark and leaves of *Commiphora swynnertonii* were tested against fungi and bacteria using agar well diffusion method. The fungi included *Candida albicans* and *Aspergillus niger* whereas the bacteria species included *Staphylococcus aureus, Streptococcus pyogenes, Bacillus subtilis, Pseudomonas aeruginosa, Salmonella typhimurium* and *Escherichia coli*. Antimicrobial activity was determined by measuring inhibition zone diameters around agar wells. Resin and root bark extracts showed significant activities against *S. pyogenes, E. coli* and *B. subtilis* compared to the other two extracts. Growth of *E. coli* was highly reduced by resin extract (minimum inhibition concentration = 1.9 µg/ml). The fungi, *C. albicans*, also showed similar sensitivity to resin and root bark extract. Growth of *S. typhimurium* was not reduced by all four extracts at all concentrations tested whereas that of *P. aeruginosa* was slightly reduced. Cytotoxicity studies using brine lethality test indicated that root bark, stem bark and resin extract had effect to brine shrimps with LC_{50} of 3.5, 13.0 and 15.8 µg/ml, respectively. The current results indicate that resin and root bark extracts of *C. swynnertonii* have strong antimicrobial activity against most of the tested microbes and support the traditional use of the plant in treating various infectious diseases. Further studies are suggested to validate the use of this plant against the diseases caused by the tested microbes.

Key words: *Commiphora swynnertonii,* antibacterial activity, antifungal, cytotoxicity test, crude plant extracts.

INTRODUCTION

The use of medicinal plants and search for new drugs and dietary supplements from plants has been accelerated in recent years. Occurrence and spread of microbes resistant to conventional drugs and consumers concerns over chemical residues in foodstuff and the environment are among main factors behind this trend (Kone et al., 2004; Sibanda and Okoh, 2008; Cowan, 1999). Increased dependency on plants as an alternative for the treatment of various diseases in humans and animals by some communities has also been fuelled by unaffordable cost and unavailability of the conventional drugs (Kone et al., 2004; Sibanda and Okoh, 2008). A number of tropical tree families has been documented as having medicinal values and are therefore used widely for that purpose (Ruffo et al., 2002; Saimo et al., 2003). *Commiphora swynnertonii*, a Burseraceae family member, is among such trees. The latter is widely distributed in Africa and Asia and is among the plant species commonly used in Tanzania for treatment of dysentery and the sap of this plant is applied on animals for control of ticks, fleas and tsetse flies (Minja, 1999).

In Asia, *Commiphora* species have been used as antibacterial, anti-inflammatory, anticancer and antiviral agent (Paraskeva et al., 2008; Rahman et al., 2008). Although several scientific studies have been carried out to assess and validate the medicinal properties of *Commiphora* species (Aliyu et al., 2002; Akor and Anjorin, 2009; Musa, 2008; Paraskeva et al., 2008), only scanty information on *C. swynnertonii* is available in the literature. *In vitro* studies by Sambuta and Masola (2006) and Kaoneka et al. (2007) demonstrated anti-ectoparasitic effect of *C. swynnertonii* against ticks, mites and fleas. The use of *C. swynnertonii* on treatment of infectious diseases has so far not been evaluated. This

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paper reports on the work carried out to investigate the antibacterial and antifungal effect of different crude extracts of *C. swynnertonii* against selected bacteria and fungi of public health importance.

MATERIALS AND METHODS

Test plant and sample collection

The test plant was sourced from the Northern Tanzania District of Simanjiro (4° 0' 0 S, 36° 30' 0 E; 1360 above sea le vel). The plant was identified by a botanist as *C. swynnertonii*; a voucher specimen (reference number CK 6489) was prepared and preserved at Tanzania National Herbarium, in Arusha (Kayombo, 2009 Personal communication). Different morphological parts namely, leaves, stem barks, root barks and resin of the plant were freshly collected from the area and transported to Sokoine University of Agriculture for preparation, extraction and testing.

Preparation and extraction of plant materials

In the laboratory each morphological part of *C. swynnertonii* was handled separately. The materials were cleaned of debris using running tap water; barks were first peeled from stem/root stumps and chopped into small pieces before sun drying. The dried materials were then ground to pass through 0.1 mm sieve size using a laboratory mill (Christy Hunt Engineering Limited England) and then stored in airtight bags in a cool dry room until used. Solvent extraction was carried out according to a method described by Parekh and Chanda (2006) with some modifications. Exactly 500 g of ground plant material were soaked in 1,000 ml of ethanol (99.8% v/v) in a conical flask plugged with aluminium foil and kept for 72 h in a dark place at a room temperature. After soaking, the suspensions were filtered (Whatmann® filter paper No. 1) and the filtrate was concentrated on water bath at 50℃ using a rotary evaporator (BUCHI, Switzerland) until all the ethanol was cleared. The resin material was treated differently in that after soaking it was immediately concentrated using the rotary evaporator. The resulting crude extracts were then stored at 4℃ in airtight bot tles until used in an antimicrobial growth inhibition assay.

Test organisms

Standard and locally isolated strains of bacteria and fungi were tested. The standard strains included, *S. aureus* (ATCC 259230), *S. typhimurium* (ATCC 259525), *E. coli* (ATCC 259523) and *C. albicans* (DSM 1665), whereas local strains from our laboratory included *S. pyogenes*, *B. subtilis*, *P. aeruginosa* and *A. niger*.

Agar well diffusion assay

The standard agar well diffusion assay was used to test the ability of different crude extracts to inhibit microbial growth. 20 milliliters of molten Muller- Hinton (MH) and Saboroud dextrose agar (SDA) were poured on sterile Petri dishes and incubates at 37℃ overnight to confirm sterility. The bacterial and fungal isolates were serially diluted into inoculums of 1×10^{-2}Cfu, streaked into MH plates and left for about 30 min to dry (Ayo et al., 2007). After allowing the plates to dry, 6 mm diameter wells were punched on each agar plate. The wells were numbered accordingly to match with the code number of test extract concentrations. Gentamycin (antibiotic) and Ketoconazole (antifungal) were used as positive control whereas dimethylsulphoxide (DMSO) was a negative control. Stock crude extract solutions were prepared by dissolving 5 g of the crude ethanolic extracts in 5 ml DMSO. A serial dilution method was then used to prepare the working solution of five different concentrations of 0, 50, 100, 150, 200 and 500 mg/ml. Then, 100 µl of each extract at different concentrations was poured into wells in quadruplicates and marked accordingly. The assembly was allowed to settle for about 15 min before the plates were incubated at 37℃ for 24 h. Assessment of antimicrobial activity was based on measurement of the diameter (mm) of inhibition zones formed around the wells. The inhibition zone diameter measurements were interpreted as follows: 6 mm = no inhibition, 7 to 10 mm = weak activity, 11 to 13 mm = moderate activity and > 14 mm = strong activity.

Minimum inhibitory concentration (MIC)

The MIC that is, the lowest concentration of a compound that inhibits growth of a microorganism was only evaluated on plant extracts which showed some antimicrobial activity using the agar diffusion assay. MIC was determined by the standard two-fold dilution technique using micro-dilution technique with nutrient broth medium as described by Obi et al. (2007).

Brine shrimp lethality assay of the study plants

Assessment of the crude extracts toxicity was done using the standard brine shrimp *(Artemia salina)* lethality test as described by Meyer et al. (1982).

Statistical analysis

The results were analyzed using Micro Soft Excel statistical package (2007). Means and standard deviation were determined. Analysis of variance (ANOVA) and Student t-test were used to compare mean values among different experimental groups whereby *P*-values of 0.05 or less were considered significant. The mean mortality of brine shrimp against the logarithms of concentrations was plotted using the KaleidaGraph Synergy Statistical package which also gives the regression equations. The regression equations were used to calculate LC_{50} values as well as confidence intervals at 95%. Extracts giving LC_{50} values greater than 20 µg/ml were considered to be non-toxic.

RESULTS

The effect of various crude extracts from *C. swynnertonii* on growth of on various microbes, as measured by the agar well diffusion assay, is shown in Table 1. A dose-response relationship was clearly evident (combined R^2 = 99.92; $p < 0.001$) in all extracts and microbial species tested. Generally, gram positive bacteria showed significantly higher ($p < 0.01$) growth inhibition zones than their gram negative counterparts although a gram negative bacterium, *E. coli* had the highest inhibition zone of all organisms tested (Figure 1). Resin and root bark extracts showed strong growth inhibition activity ($p < 0.001$) against *S. pyogenes, E. coli* and *B. subtilis* with inhibition zones of 23.3 ± 3.9, 21.5 ± 4.8 and 15.7 ± 3.0 mm, respectively compared to the other two extracts. *P. aeruginosa* and *S. typhimurium* were only slightly inhibited by all tested extract at the maximum

Table 1. Growth inhibition zones of various extracts from *C. swynnertonii* on growth of different microbes (means ± SD, n = 4).

Microbe	Inhibition zones (mm *)			
	Resin	Root bark	Stem bark	Leaf
E. coli	21.5 ± 4.8	17.5 ± 2.1	15.2 ± 1.6	13.6 ± 1.4
S. pyogens	23.3 ± 3.9	14.2 ± 1.8	15.1 ± 1.6	13.3 ± 3.4
B. subtilis	15.7 ± 3.5	15.6 ± 3.1	14.1 ± 2.9	12.9 ± 2.7
C. albicans	14.4 ± 4.7	18.4 ± 4.2	12.5 ± 3.4	9.4 ± 3.2
S. aureus	11.5 ± 2.9	16.3 ± 3.6	10.6 ± 2.8	8.8 ± 2.3
A. niger	12.3 ± 1.2	12.0 ± 0.9	11.7 ± 0.8	11.5 ± 1.4
P. aeruginosa	9.0 ± 2.1	10.5 ± 3.3	7.7 ± 2.5	7.0 ± 1.4
S. typhimurium	7.8 ± 2.7	8.0 ± 2.9	6 .0 ± 0.0	6.0 ± 0.0

*Diameter of disk = 6.0 mm; 6 mm = no inhibition; 7 to 10 mm = weak activity; 11 to 13 mm = moderate activity and > 14 mm = strong activity.

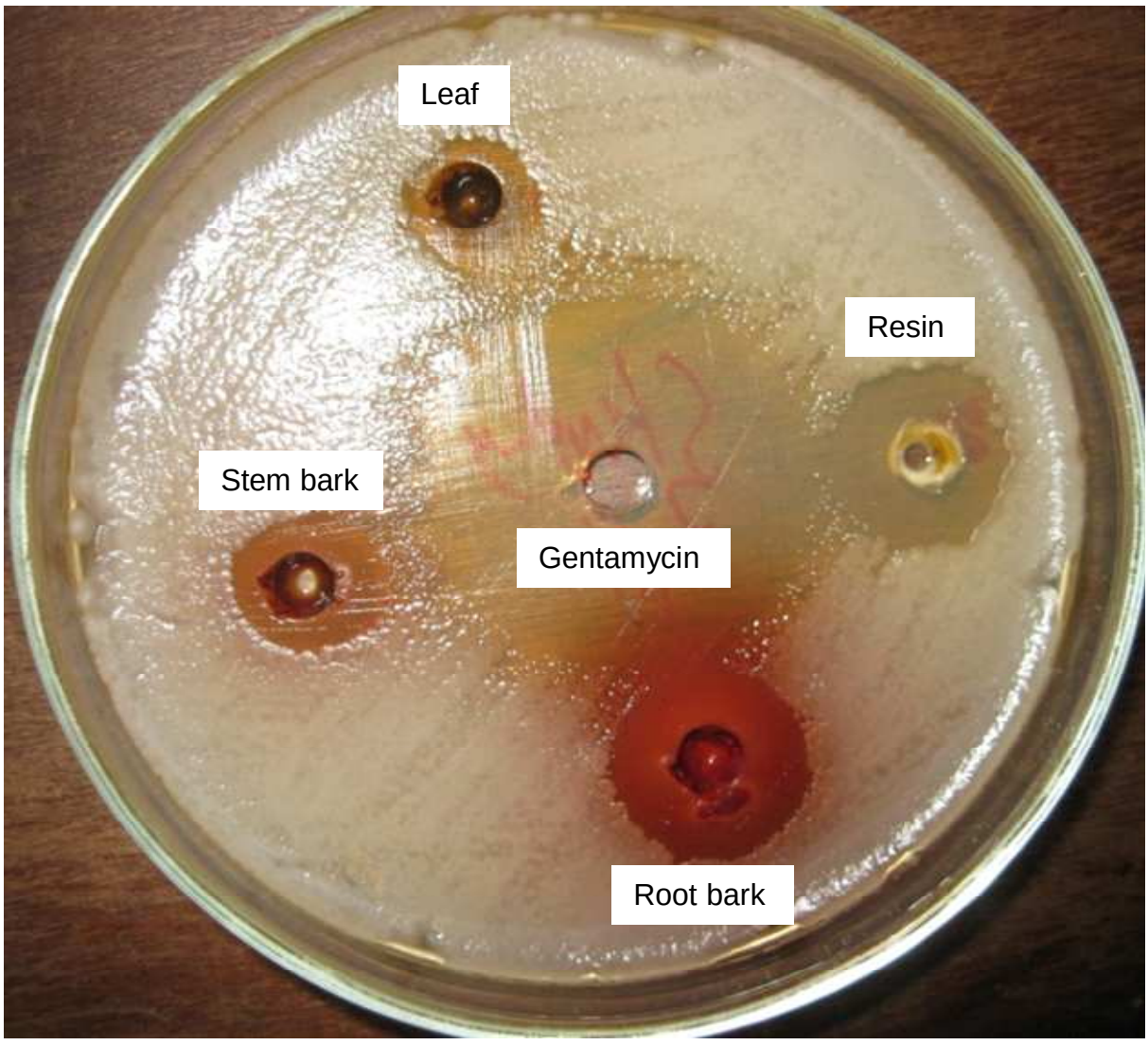

Figure 1. Growth inhibition zones of various plant extracts against *S. aureus.*

Table 2. Minimum inhibitory concentrations of various plant extracts against selected microbes.

Microbe	MIC in µg/ml			
	Leaf	Stem bark	Root bark	Resin
E. coli	> 500	250.0	62.5	1.9
S. pyogens	125.0	31.5	7.8	3.9
B. subtilis	125.0	62.5	31.5	7.8
C. albicans	> 500	62.5	3.9	31.5
S. aureus	> 500	125.0	31.5	125.0
S. typhimurium	> 500	> 500	250.0	250.0

Table 3. Cytotoxicity of extracts from *C. swynnertonii* using brine shrimp lethality test.

Plant part	LC_{50} (µg/ml *)	Confidence interval (CI)*[a-b]
Root bark extract (RB)	3.5	3.4 - 3.6
Stem bark extract (SB)	13.0	9.4 -17.9
Resin extract (RE)	15.0	10.4 - 23.9
Leave extract (LE)	96.0	62.7 - 146.9

* LC_{50} is defined as the concentration which resulted in a 50% mortality of brine shrimp; n = 10 *[a-b] Lower limit confidence - upper limit confidence interval.

concentration of 500 mg/ml. The fungi, *C. albicans* and *A. niger*, were moderately inhibited. Leaf extract gave the lowest inhibition activity to all tested microbes compared to the remaining extracts (ranking: resin > root bark > stem bark > leaf). For the two tested fungi, resin and root bark extracts showed moderate activity against *C. albicans* (Table 1). Results of MIC are shown in Table 2. Resin extract inhibited the growth of *E. coli, S. pyogenes* and *B. subtilis* at the minimum concentration of 1.9, 3.9 and 7.8 µg/ml for respectively. *S. typhimurium* was the least inhibited by the four extracts at the MIC of 250 µg/ml. The brine shrimp toxicity assay results are shown in Table 3. Leaf extract had the highest LC_{50} value (greater than 20 µg/ml) whereas root bark showed the lowest value followed by stem bark and resin extract.

DISCUSSION

Results of the current study have clearly shown that crude extracts from different morphological parts have varying antimicrobial activity *in-vitro* in a dose dependent manner. Our findings are in agreement with previous studies done by El-Ashry et al. (2003) and Abdallah et al. (2009) who found that several *Commiphora* species had considerable antimicrobial activity against some gram positive and gram negative bacteria. Furthermore, *in-vitro* studies by Paraskeva et al. (2008) using selected South African *Commiphora* species, showed more activity against gram positive bacteria than gram negative. Similarly, in the current study, gram positive bacteria showed significantly higher ($p < 0.01$) growth inhibition zones than their gram negative counterparts although a gram negative bacterium, *E. coli* was most sensitive to the four extracts compared to all organisms tested. The difference in susceptibility between gram positive and gram negative has been associated with their cell wall structure (Parekh and Chanda, 2007). Gram–negative organisms are considered to be more resistant due to their outer membrane / cell wall acting as a barrier to many environmental substances, including antibiotics. Resin extract ranked the highest in inhibiting the growth of the tested microbes; with largest inhibition zones against *S. pyogenes, E. coli* and *B. subtilis* in that order. Similar studies using *C. quadricincta* (Salamah and Zaid, 1999) also showed higher activity of resin against the three bacteria in comparison to other extract tested. Akor and Anjorin (2009) reported highest activity of *Commiphora africana* against *E. coli*, *S. aureus* and *C. albicans*. Also Musa (2008) demonstrated a good activity of *Commipora kerstingii* against *S. aureus*. Furthermore, Akor and Anjorin (2009) reported that *E. coli* and *B. subtilis* were the most susceptible among microorganism treated with crude ethanolic root extract from *C. africana. S. typhimurium* and *P. aeruginosa* were the least affected by the crude extracts. Resistance of these two bacteria to crude plant extracts and even commonly used antibiotics has been documented in other studies. Parekh and Chanda (2007) tested twelve species of Indian medicinal plants and found that *S. typhimurium* and *P. aeruginosa* were resistant to all tested plants. Also, *P. aeruginosa* was shown to be resistant to root extract of *C. africana* (Musa, 2008; Akor and Anjorin, 2009). Resistance of the two bacteria to various antibiotics has been reported by

Brisabois et al. (1997) and Wang et al. (2006). This resistance was associated with presence of resistant genes, PSE and CARB-type, in both the bacteria and animals.

These genes are located on an integron, a new family of genetic components into which many resistance agents can fit (Brisabois et al., 1997). The antibacterial activity of various *Commiphora* spp. has been attributed to presence of different active constituents. The commonly reported active constituents include phenolic compounds, alkaloids, saponins, tannins, flavonoids, anthraquinones and cardiac glycosides, terpenes, sesquiterpenes, esters cumunic aldehyde, eugenol, steroids, resin acids and proteins (Hanus et al., 2005; Aliyu et al., 2007; Musa, 2008; Abdallah et al., 2009). The antibacterial activity of *Commiphora molmol* was attributed to presence of terpenes in its oleo-resin (Rahman et al., 2007). The brine shrimp lethality assay was carried out to assess toxicity of extracts from different morphological parts of *C. swynnertonii*. Results from this study indicated that all tested extracts (with exception of leaf extract) had LC_{50} values below 20 µg/ml suggesting that exposure to high concentrations can be acutely toxic to biological systems.

Brine shrimp LC_{50} values of medicinal plants have been used to predict anti-carcinogenic activity when values are less than 20 µg/ml (Meyer et al., 1982). The current study has clearly demonstrated that crude extracts, especially resin, from *C. swynnertonii* have varying degrees of antimicrobial activity. The resin extract, apart from being the most potent, seems to be more appropriate because it's harvesting causes minimal damage to the plant and also showed less cytotoxic effect than the root and stem barks. These findings support the traditional use of the resin in treatment of various infectious diseases. Further, *in vivo* investigations using the resin are recommended so as to validate the use of *C. swynnertonii* as an antimicrobial agent against infectious diseases caused by the tested pathogens.

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