Antimicrobial screening of crude extracts from the indigenous *Ganoderma lucidum* mushrooms in Namibia

L. T. Shikongo¹², P. M. Chimwamurombe¹*, H. R. Lotfy² and M. Kandawa-Schulz²

¹Department of Biological Sciences, University of Namibia, Private Bag 13301 Windhoek, Namibia.
²Department of Chemistry and Biochemistry, University of Namibia, Private Bag 13301 Windhoek, Namibia.

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The indigenous *Ganoderma* mushroom belongs to the class Basidiomycetous. It has been used in different systems of traditional medication for the treatment of diseases of human beings and animals. It contains various triterpenes, polysaccharides, alkaloids and steroids known to have broad effects pharmacologically and anti-bacterial properties. The indigenous *Ganoderma* mushroom has been used by locals in Namibia traditionally as a source of medicine to fight skin and wound infections, and other ailments. There is a need to validate the traditional usage of *Ganoderma* mushroom extracts on *Escherichia coli*, *Alcaligenes faecalis*, *Proteus vulgaris*, *Neisseria meningitidis*, *Bacillus cereus* and *Staphylococcus aureus*. This is important for the Namibian local communities, where people do not have access to modern medicines and use traditional medicines. This study analyzed the anti-bacterial effects of crude organic and aqueous extracts of the mycological components of the indigenous *Ganoderma* mushroom. Using the agar disc diffusion method, the crude extracts of the *Ganoderma* mushroom exhibited various degrees of inhibition against the tested organisms. The widest inhibitory zones (19.0 mm) were obtained with the crude benzene extract of *G. lucidum* against *E. coli* and *N. meningitidis*. The lowest zone of inhibition (6.0 mm) was demonstrated with the aqueous extract against *E. coli*. The study has concluded that the crude extracts of the indigenous Namibian *Ganoderma* mushroom possess antibacterial properties to all Gram positive and negative strains tested.

Key words: *Ganoderma lucidum*, antibacterial properties, *Escherichia coli*, *Alcaligenes faecalis*, *Proteus vulgaris*, *Neisseria meningitidis*, *Bacillus cereus* and *Staphylococcus aureus*.

INTRODUCTION

In recent years, there have been a significant number of human pathogenic bacteria becoming resistant to antimicrobial drugs (Donadio et al., 2002) and this is in part due to the misuse and overuse of available antibiotics (Monroe and Polk, 2000). Antimicrobial drug resistance is of major economic concern and impacts on physicians, patients, health care administrators, pharmaceutical producers and the public (McGowan, 2001). In addition, bacterial and fungal pathogens have complicated the treatment of infectious diseases (Baratta et al., 1998). Given the increase in multiple drug resistance of human pathogenic microorganisms, it is imperative that new and effective therapeutic agents be developed.

Traditional tribal communities, indigenous peoples and the eastern world have been using plants, spices and fungi for thousands of years as therapeutic agents. For the past decades, attention has turned to extracts and biologically active compounds used in traditional herbal medicine to uncover the scientific basis of their remedial effects and to seek new lead compounds for development into therapeutic drugs (Cragg et al., 1997). In addition to plants extracts as sources of antimicrobial
agents, research is being performed on fungi for their ability to mobilize the body's humoral immunity and in turn prevent bacterial, viral, or fungal pathogens that are resistant to current therapeutic agents (Wasser and Weis, 1999c). Fungi are well known for the production of important antibiotic compounds such as penicillin, however, the occurrence of antibiotics in the class of fungi known as Basidiomycetes (the mushrooms) is scarcely documented (Miles and Chang, 1997) and there are only few reviews that summarize the antibacterial activity from these organisms (Gao et al., 2003; Zjawiony, 2004). Ganoderma species belong to the Basidiomycetes class of mushroom, which is known to possess a variety of biochemical compounds with a wide range of pharmacological effects. Ganoderma species have been widely investigated for their therapeutic properties as antitumor and antiviral agents but have been far less investigated as a source of new antibacterial agents. A review by Gao et al. (2003), on the antibacterial and antiviral value of Ganoderma species supported this observation.

In Namibia, especially in the northern and northeastern part, Ganoderma have been used in relieving stress when sniffed as ash mixed with tobacco, calming of nerves when put in water, used as a drink. It is also used in the treating of cold and flu symptoms when its smoke is inhaled. Its extracts are applied to infected skin and to treat the wounds on children's heads. Kadhila-Muandingi (2010) and Ekandjo and Chimwamurombe (2012) reported the same traditional uses of Ganoderma mushroom in Oshangwena and Oshana regions of Namibia. Yongabi et al. (2004) also reported the use of crushed Ganoderma mushroom mixed with ash as ointment in the treatment of skin infections in Cameroon.

Locally, this mushroom is also said to have a history in treating animal diseases, especially cattle when suffering from lung diseases and goats with skin rashes (Kadhila-Muandingi, 2010; Yongabi et al., 2004).

There appear to be an increasing number of reports on Gram-positive bacteria developing resistance to virtually every clinically available drug (Donadio et al., 2002), and Basidiomycetous mushrooms have been shown to possess antibacterial activity against this group of bacteria. Other work showed that extracts were also active against Gram-negative organisms, Proteus vulgaris and Escherichia coli, in vitro (Yoon et al., 1994). Overall, extracts from mushrooms are observed to be more active against Gram-positive bacteria than Gram-negative bacteria (Smania et al., 1999).

It should be noted that the indigenous knowledge of medicinal mushroom use is linked to local culture and history (Opige et al., 2006). To our knowledge no studies have been conducted to validate the assessment of traditional medicinal uses of the indigenous Ganoderma mushrooms in Namibia. For this reason, this study was carried out to determine the antimicrobial properties of the aqueous and organic crude extracts from the fruiting bodies of the indigenous Ganoderma mushroom against E. coli, Staphylococcus aureus, Bacillus cereus, Neisseria meningitides, Alcaligenes faecalis, Proteus vulgaris which are known to cause wound infections, intestinal and urinary-genital tract infections, skin infections and abdominal cramps/diarrhea.

**MATERIALS AND METHODS**

**Sample collection**

Ganoderma lucidum (Picture 1) samples were collected from the natural environment of the northern and northeastern part of Namibia (namely Oshana, Ohangwena, Kavango and Caprivi regions). The mushroom fruiting bodies were identified by its shelf-like with a short stalk, and leathery to corky when fresh. They were present singly or in overlapping clusters at or near ground level. The upper surface is dark reddish brown and has a thin, shiny, varnish-like crust that becomes coated with a layer of dull brown basidiospores. The pore surfaces are creamy white, becoming light buff and bruising dark brown, according to Van der Westhuizen and Eicker (1994).

**Organic and aqueous extraction**

The anti-bacterial compounds were extracted from Ganoderma mushroom samples with aqueous and organic solvents, in order to separate the chemical constituents into groups of different polarities. First, a "successive step extraction" was applied to determine the polarity of the mycochemical antibacterial compounds. Different solvents used present different polarity in order to extract successively compounds of different polarities: lipids, sterols, triterpenoids, glycoproteins, glycosides, sugars, amino acids and proteins. The solvents were used in order of increasing polarity: Benzene, chloroform, ethyl acetate (EA), ethanol (EtOH), methanol (MeOH) and aqueous extraction as hot water (HW) extract. All reagents were of analytical grade and were used as received.

About 45 g of each powdered sample was soaked and cold extracted with 400 mL of the organic solvents at room temperature for 5 days. The organic solvents were used successively with gradient polarity starting with benzene, chloroform, EA, EtOH, MeOH and aqueous extraction (HW). The crude extracts were gravity filtered through a 0.45 μm Whatman No. 2 filter paper. The filtrates were concentrated by evaporating excess solvent in a hot water bath and stored in the dark.

**Picture 1. Ganoderma lucidum.**
Figure 1. Positive zones of inhibition of (A) crude EA Ganoderma extracts against P. vulgaris, (B) crude methanol Ganoderma extracts against E. coli, (C) and (D) crude EA Ganoderma extracts against E. coli and B. cereus. Pure solvents were used as positive controls and no inhibition was observed.

Culturing of microorganisms and antibacterial testing

Lyophilized test microorganisms: E. coli (ATCC 25922), S. aureus (ATCC 25923), B. cereus (ATCC 10876), N. meningitides (Y) (ATCC 35561), A. faecalis (ATCC 8750), P. vulgaris (ATCC 33420) were obtained from the University of Namibia Microbiology Laboratory. They were grown on Nutrient Broth and further grown on Mueller-Hinton agar (MHA). All microorganisms were grown at 37°C. A disc-diffusion method was used to determine the antimicrobial activity of the crude Ganoderma mushroom extracts as described by Kisangau et al. (2007). Paper discs (6 mm, diameter) were dipped into the mushroom crude extracts using sterilized forceps and placed on the surface of the inoculated Petri dishes (Kisangau et al., 2007). The plates were incubated at 37°C for 24 h and observed for zones of inhibition. The anti-bacterial activity of each extracts was recorded by measuring any zone of growth inhibition diameter around the disc with a millimeter ruler. The experiments were done in triplicate and the average values were tabulated.

RESULTS

Antimicrobial sensitivity test

The tests revealed that the crude extracts of the indigenous Ganoderma mushroom of all different polarities as presented by the solvents used contain antimicrobial effects. The solvent used for each extraction was used as the negative control for each extract. The qualitative and quantitative analysis of each crude extract will be published once available in a separate publication.

DISCUSSION

Results from the tests indicate the presence of more than one mycochemical class of compounds of different polarities having antibacterial effects on the microbial strains tested. The inhibitory activities of all crude organic and aqueous extracts (Figure 1) were investigated against Gram-positive and Gram-negative bacteria. The results indicated that both aqueous and organic extracts from Ganoderma possessed activities against all tested microbial strains. The results presented in Table 1 show the inhibition zones of crude organic and aqueous extracts against all the microbial strain tested. Benzene crude extracts showed the largest inhibition zone as compared to other crude organic solvents against E. coli, S. aureus, B. cereus, N. meningitidis, A. faecalis and P. vulgaris.
The maximum inhibition zone of the crude aqueous extract of the *G. lucidum* fruit bodies obtained was 18.0 mm against *N. meningitidis* and minimum of 8.0 mm against *E. coli* and *P. vulgaris*. Whereas the maximum inhibition zone of crude organic solvents extracts against the test microbial strains obtained was 19.0 mm against *E. coli* and *N. meningitidis* of benzene extracts, and a minimum of 6.0 mm of benzene extracts against *A. feacalis*, 6.0 mm of chloroform extracts against *P. vulgaris* and *A. feacalis*, 6.0 mm of EA extracts against *S. aureus*, and 6.0 mm of MetOH extracts against *A. feacalis*. Crude benzene, EA extracts appeared to have the strongest antibacterial effect therefore a larger inhibition zone. It could be that benzene extracted most of the non-polar components while EA extracted most of the polar components of the mushrooms thus exerted the most antimicrobial effects.

This is in agreement with activities observed for the benzene extracts against the tested microorganisms of the Gram-positive bacteria *B. cereus* (Roberts, 2004). Also in agreement with reports of Ofodile et al. (2005) that aqueous extracts of *Ganoderma* exhibited inhibitory activity towards the *Bacillus* species.

It showed be noted that the organic solvents used for extraction were assayed as negative controls, of which no inhibition zone activities were recorded and observed against the tested microbial strains.

The different antibacterial activities observed may be due to different mycochemical compounds which were detected in the fruiting bodies of the indigenous *Ganoderma* mushroom. *Ganoderma* is known to possess various chemical compounds such as triterpenoids, flavanoids, coumarins, quinones, carotenoids and amino acids as having antibacterial properties (Roberts, 2004).

There have been reports showing that triterpenes have a great antibacterial effect (Wilkens et al., 2002) and it is well documented that triterpenes are one of the major constituents isolated from *Ganoderma*.

**S. aureus** is an important causative agent of invasive skin diseases including superficial and deep follicular lesion (Usman et al., 2007). Therefore, antibacterial activity detected in the benzene crude extracts of the indigenous *Ganoderma* mushroom against *S. aureus* and *P. vulgaris* support the use of this indigenous *Ganoderma* mushroom to treat skin and wound infection as indicated by local people.

**Conclusion**

This study demonstrated that the indigenous *Ganoderma* mushrooms may have a good potential for the production of useful bioactive metabolites and they may serve as a good source for antimicrobial drugs. Effective concentrations of the active organic extracts were not investigated in this study and will be published once available in a separate publication. This study provides justification to conduct further research to evaluate and characterize the antibacterial activity of the indigenous *Ganoderma* mushroom extracts on a wider range of clinically relevant microbes.

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