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Antimicrobial activity of three Namibian ethno-medicinal plants:!Guxa (*Aptosimum albomarginatum* Marloth and Engl.), Aru (*Albizia anthelmintica* A. Rich Brongn.) and Gu-!aru (*Dicoma schinzii* O. Hoffm.)

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Keywords: *Aptosimum albomarginatum, Albizia anthelmintica, Dicoma schinzii,* traditional ethno-medicinal plants, antimicrobial activity, Namibia.

Abstract

With antibiotic resistance being an enduring problem worldwide, alternative treatment options, such as 'natural antibiotics', should be considered. This work aimed to perform antimicrobial assays, with the purpose of scientifically evaluating the effectiveness of three Namibian ethno-medicinal plants, namely:!Guxa (*Aptosimum albomarginatum* Marloth and Engl.), Aru (*Albizia anthelmintica* A. Rich Brongn.) and Gu-!aru (*Dicoma schinzii* O. Hoffm.). It was found that !Guxa root extract exerted antimicrobial activity against five out of the eight strains tested, with strong activity against *Streptococcus sanguinis* at an extract concentration of 20mg/ml. Moderate activity was observed for the other cultures tested. *Candida albicans, Escherichia coli* and *Pseudomonas aeruginosa* were resistant

to !Guxa. These results give some scientific proof regarding the usefulness of !Guxa root extract as traditional medicine in some instances, thereby generating new knowledge to be shared with fellow researchers.

Abstrak

Met antibiotiese weerstand wat tans ’n blywende wêreldwye probleem is, moet alternatiewe opsies van behandeling, soos ‘natuurlike antibiotika’, oorweeg word. Hierdie werk het beoog om antimikrobiese toetse te doen, met die doel om die effektiwiteit van drie Namibiese etno-medisinale plante, naamlik: !Guxa (*Aptosimum albomarginatum* Marloth and Engl.), Aru (*Albizia anthelmintica* A. Rich Brongn.) en Gu-!aru (*Dicoma schinzii* O. Hoffm.), wetenskaplik te evalueer. Daar is bevind dat !Guxa wortelekstrak antimikrobiese aktiwiteit getoon het teen vyf uit agt stamme getoets, met sterk aktiwiteit teen *Streptococcus sanguinis* by ’n konsentrasie van 20mg/ml. Matige aktiwiteit het voorgekom by die ander kulture wat getoets is. *Candida albicans*, *Escherichia coli* en *Pseudomonas aeruginosa* was weerstandbiedend teen !Guxa. Hierdie resultate bewys tot ’n mate wetenskaplik dat !Guxa wortelekstrak bruikbaar kan wees as tradisionele medisyne in sommige gevalle en het gevolglik tot nuwe kennis gelei om met mede-navorsers te deel.

Introduction

Antibiotic resistance is currently a worldwide problem. Therefore, scientists should investigate alternative treatment options, such as the use of natural products. Plant-derived products may have antimicrobial properties, acting as ‘natural antibiotics’. Such natural products may in some instances also be used as adjuvants together with antibiotics.

Most cultures across the globe have developed knowledge of local plants, enabling them to use these plants for medicinal purposes (Silvério & Lopes, 2012: 110–11). Certain plants utilized in some regions are unknown to western medicine and thus studied in the field of ethnopharmacology to investigate their antimicrobial properties. The concentration of pharmacologically active compounds depends on the season during which the plants are harvested, how mature they are when harvested, and the conditions under which growth has taken place. Due to lack of regulation, the same plant product bought at different times can possess different biological properties (Silvério & Lopes, 2012: 110–11).

In the traditional setting, especially in rural areas, plants are used as natural medicine to treat various illnesses, including bacterial and viral infections. According to van Wyk & Wink (2015: 276, 284) bacteria are microorganisms consisting of a single cell surrounded by cell walls; their DNA is circular and they do not have internal membrane systems or nuclei. Viruses on the other hand, are infectious complexes of macromolecules with their genetic information as either DNA or RNA. Viruses require host cells for replication and

formation of new viral particles. *Aptosimum albomarginatum* (Marloth and Engl.) shown in Figure 1 (A) is commonly known as '!Guxa' by the Nama tribe in Namibia. The roots are pulverized, boiled as a tea, and drunk to purify the blood and cleanse the uterus. Some believe that it can cure women who experience difficulty in conceiving (S. Coetzee, personal communication, February 2015; A. Frederick, personal communication, February 2015). Staphylococcus bacteria may be associated with infection of the uterus, for example in the medical condition known as endometritis (inflammation of the endometrium). One form of this condition is known as bacteriotoxic endometritis, where it is caused by the toxins of bacteria rather than the presence of the pathogens themselves (*Dorland's illustrated medical dictionary,* 2003: 614). The prepared !Guxa tea also helps to relieve the symptoms of colds (S. Coetzee, personal communication, February 2015; A. Frederick, personal communication, February 2015). Colds are due to viruses, not bacteria. However, when one sneezes as a result of cold symptoms, lots of bacteria in the nose can quickly spread to one's surroundings and other people (Bischoff et al., 2006: 1119).

Albizia anthelmintica (A. Rich Brongn.) in Figure 1 (B) has many common names in different languages, including kersieblomboom, worm-cure albizia (Orwa et al., 2009: 1; Hoffmann, 2014), aruboom, oumahout, Wurmindenbaum, Kirschblütenbaum and omuama. According to local Nama people at Gochas, the outer part of the twigs is scraped off and the inner part is used as a chewing stick or toothbrush to clean the teeth and tongue (S. Coetzee, personal communication, February 2015; A Frederick, personal communication, February 2015). Bacteria and fungi may be associated with dental plaque and mouth infections. The bark, wood or root is boiled and milk added to treat an upset stomach or intestinal worms. Tea made from the roots and bark is drunk to treat malaria. The Samburu pastoralists in Kenya treat gonorrhoea by boiling the roots, bark and leaves, mixing it with sheep fat and giving it as an enema. Otherwise the boiled bark and roots are consumed with milk (Sullivan, 1998: 46; du Pisani, 1983; Fratkin, 1996: 75). The boiled bark, wood and roots can also be used to de-worm livestock (Fratkin, 1996: 81). The stem bark is widely used as a purgative (Orwa et al., 2009: 3).

Dicoma schinzii (O. Hoffm.) in Figure 1 (C) is also known as "Gu-!aru" in the Nama language (S. Coetzee, personal communication, February 2015; A. Frederick, personal communication, February 2015) or the 'Kalahari fever bush' (Dugmore & van Wyk, 2008). The roots and leaves are pulverized, boiled as tea and drunk or used to steam oneself in the treatment of measles, chickenpox, influenza, colds, and a blocked nose (Coetzee, 2015). These are viral infections, but bacteria such as staphylococci can be involved in congested nose or sinus infections. According to van Wyk & Gericke (2000) and Dugmore & van Wyk (2008) unspecified parts are used to treat febrile convulsions in babies in the Kalahari, hence the name 'Kalahari fever bush'.

There is an interesting folk tale ('Dicoma's shadow') behind the plant's traditional use in the Kalahari to treat febrile convulsions in babies. Van Wyk (2015) relates the story in short: It is said that if the shadow of the black shouldered kite (*Elanus caeruleus*) falls on a baby, the child will get sick, and the illness will be recognized by the spastic movements

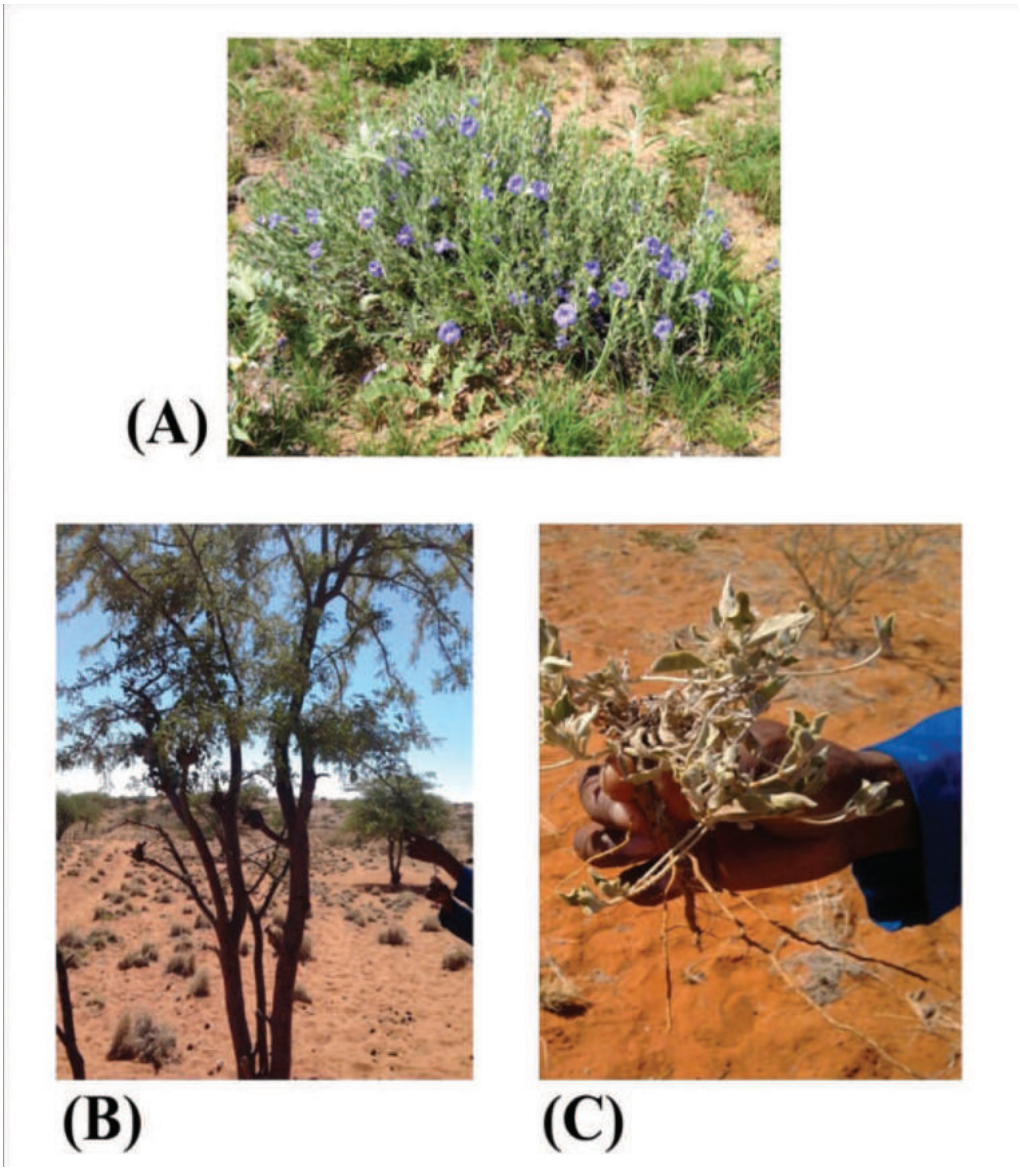

Figure 1 A. albomarginatum *(A). Image credit: southafricanplants.net;* A. anthelmintica *(B), and* D. schinzii *(C) growing in the veld at Gochas. Image credit: Sunette Walter.*

of the baby's arms, similar to the movements made by the bird's feathers when it is hovering above its prey. If the condition is not treated the infant can develop feathers on the arms. An extract of the plant can be given both topically and internally to counteract the symptoms and cure the child. In the traditional African context the symbol of the bird represents fever, since birds have a higher natural body temperature (40°C) compared to that of humans (37°C). 'The condition of the bird' refers to fever – one of the symptoms of febrile convulsions in infants.

To make sure of their effectiveness and to rule out placebo effects, this work aimed to evaluate scientifically the antimicrobial activity and medicinal value of the three Namibian ethno-medicinal plants. The objective was to test five crude methanolic extracts from the plants against seven bacterial strains and a fungus, to see if the extracts could inhibit microbial growth on agar plates. Prescott, Harley & Klein (2002) describe bacteria as single-celled organisms, made up of prokaryotic cells, whereas they describe fungi as achlorophyllous, heterotrophic, spore-bearing eucaryotes with absorptive nutrition, usually with a walled thallus.

Methodology

Cultures and plant extracts used

The following cultures were supplied by the University of Pretoria's Biochemistry Department: *Escherichia coli* ATCC 700928, *Staphylococcus aureus* ATCC 12600, *S. aureus* ATCC U3300, *Bacillus subtilis* ATCC 13933, *Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Pseudomonas aeruginosa*, and *Candida albicans*. Crude methanolic extracts (prepared at the University of Namibia) of !Guxa roots, Aru leaves and twigs, and Gu-!aru roots and leaves were used.

Preparation of crude extracts

To prepare crude methanolic extracts, 10g of plant material (in dried powder form) from the different plant parts was added to 100ml methanol. Flasks containing the extracts were parafilmed, placed in a cupboard and left to stand for three days (maceration), with occasional swirling. After three days, the extracts were gravitationally filtered through Whatman 110mm filter papers. The extracts were rotary evaporated in round bottom flasks at reduced pressure (91mbar) and temperature (45°C) to evaporate the methanol, and to dry and concentrate them. To avoid thermal decomposition of compounds in the plant material, the temperature set for the rotary evaporator (Heidolph, Germany) did not exceed 45°C. The flasks were labelled, sealed with parafilm, and kept at -86°C for a few hours. Thereafter, the frozen extracts were connected to a Christ Alpha 1-2 LD Plus freeze-dryer (Germany) for two to four days to further dry and concentrate them. Dried extracts were

scraped off with a spatula, weighed and stored in labelled 50ml centrifuge tubes, and kept at -86°C for further use.

Antimicrobial assays

Antimicrobial assays were performed according to the methodology described by Beukes (2015). Overnight cultures were grown in eight Eppendorf tubes each containing 1ml of brain heart infusion broth. Brain heart infusion agar was prepared, thinly poured into eight sterile 90mm petri dishes and allowed to set. Soft agar was prepared by using half the quantity of agar normally used, dispensed into eight sterile test tubes as 7ml quantities, and placed in an oven at 50°C to keep the agar molten. Plant extracts were dissolved as 20mg/ml concentrations in Eppendorf tubes containing 100% dimethyl-sulfoxide (DMSO). It was previously determined that 100% DMSO has no effect on the cultures. Tubes were vortexed to ensure that the extracts dissolved completely.

The soft agar was allowed to cool a bit, 100µl of the overnight cultures was pipetted into the soft agar, vortexed and poured as an overlay onto the brain heart infusion plates. The plates were placed in a laminar flow cabinet for half an hour to solidify. Thereafter, 10µl of each plant extract was pipetted onto the solidified agar-overlay plates (five extracts per plate). The plates were left in the laminar flow cabinet for half an hour to allow for the extracts to dry and diffuse into the agar. Finally, the plates were incubated at 37°C for 24 hours and zones of inhibition were measured to the nearest millimetre with a ruler. In cases where zones were unevenly shaped instead of circular, measurements were taken at the broadest and narrowest part and averaged.

On a second occasion, to determine the minimum inhibitory concentrations of extracts against each culture, this method was used at extract concentrations of 20, 10, 5, 2.5, 1.25 and 0.625mg/ml. Each extract was initially dissolved in 100% DMSO and subsequently diluted and vortexed in sterile triple distilled water before dripping 10µl of each concentration onto plates. The classification by Nematollahi et al. (2011) was used to interpret results: ≤ 7mm (negative), 8–10mm (weak activity), 11–14mm (moderate activity), 15–24mm (strong activity), and ≥ 25mm (very strong activity).

Results and Discussion

Antimicrobial assays

At first the five plant extracts were tested against the cultures at a single concentration of 20mg/ml. Only !Guxa roots displayed antimicrobial activity. This activity was highest in *S. sanguinis*, with an inhibition zone of 15mm (strong activity), followed by *S. aureus* ATCC 12600 (13.5mm; moderate activity), *S. mutans* (12mm; moderate activity), *S. aureus* U3300 (11.5mm; moderate activity), and *B. subtilis* (11.5mm; moderate activity).

Candida albicans, *E. coli*, and *P. aeruginosa* were resistant to !Guxa root extract, i.e., the extract was inactive or did not display antimicrobial activity against these microorganisms. Classification of activity was according to values given by Nematollahi et al. (2011).

The extracts were tested a second time at concentrations of 20, 10, 5, 2.5, 1.25, and 0.625mg/ml. Once again, only !Guxa roots displayed antimicrobial activity against the five above-mentioned strains, with no activity against the other three. With decreasing concentrations of extracts, antimicrobial activity decreased from strong (15.5mm inhibition diameter) to moderate (11mm–13mm) to weak (10.5mm) in *S. sanguinis*, moderate (11mm–12.5mm) to weak (9.5mm–10.5mm) in *S. aureus* ATCC 12600, moderate (11mm–14mm), weak (10mm) to no inhibition (0mm) in *S. mutans*, moderate (12mm) to weak (9.5mm–10.5mm) in *S. aureus* U3300, and moderate (11.5mm–14mm) to weak (10mm–10.5mm) in *B. subtilus*. *Streptococcus sanguinis*, *S. aureus* ATCC 12600, *S. aureus* U3300, and *B. subtilis* had MICs of 0.625mg/ml, while the MIC for *S. mutans* was 1.25mg/ml. Using the classification by Nematollahi et al. (2011) clinically important/usable effects of !Guxa root extracts would be at observed inhibition zones of 11mm–14mm (Moderate antimicrobial activity), 15mm–24mm (Strong activity) and ≥ 25mm (Very strong activity, not observed in this study).

Antimicrobial activity observed can to some extent be ascribed to the presence of secondary metabolites such as flavonoids, saponins and triterpenes (Walter, 2018). As mentioned in the Introduction, the concentration of pharmacologically active compounds depends on the season that the plants were harvested, how mature they were and the conditions in which growth took place (Silvério & Lopes, 2012: 110–11). These factors therefore might play a role in the biological inactivity observed with the other plant extracts.

Conclusion and Recommendations

Out of all plant extracts tested, only !Guxa root extract displayed antimicrobial activity against five out of eight strains. At a concentration of 20mg/ml this activity was highest in *S. sanguinis* (strong activity), with moderate activity against the other cultures. *Candida albicans*, *E. coli* and *P. aeruginosa* were resistant to !Guxa roots. These results give some scientific proof that !Guxa roots can be useful as traditional medicine as a natural antimicrobial agent in some instances, thereby generating new knowledge to be shared with fellow researchers. For antimicrobial activity, extract concentrations higher than 20mg/ml can be tested. Further assays should be done to determine the mode of action of the extract against the strains tested. In a doctoral study by the author (Walter, 2018) thin layer chromatography (TLC) for screening of phytochemical compounds indicated the presence of flavonoids, saponins and triterpenes in !Guxa roots. These compounds can be responsible for the roots' activity against the microorganisms. Larger, in-depth studies could look into these three compounds, as well as additional compounds. Cytotoxicity assays should also be conducted to ensure the safety of the plant and its extracts for human consumption.

As mentioned by van Wyk (2015: 10), prospective studies can look into the potential of !Guxa to be a commercial plant and a source of income for Namibians currently using it for medicinal purposes. This would however not be an easy or rapid process and its harvesting would involve permission and obtaining of permits from the relevant parties, such as the Ministry of Environment, Forestry and Tourism, as well as the farmers owning the land where these shrubs grow. With permission from the Ministry of Health, extensive clinical studies should be conducted to ensure the safety, efficacy, and potential adverse or side-effects/allergic reactions due to consumption of medicinal teas and medication or supplements manufactured from these plants or their active compounds.

Conflicts of Interests

The author declare that there is no conflict of interest.

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About the Author

Sunette Walter was born in Upington, South Africa. She holds a BSc degree majoring in Human Physiology and Microbiology (2006), BSc Honours in Water Sciences (2007), MSc in Environmental Sciences (2010), from the North-West University (NWU - PUKKE), Potchefstroom, South Africa, and PhD in Microbiology from the University of Namibia (UNAM). Her Honours study at NWU aimed to make use of cultivation-dependent methods for the isolation and characterization of bacteria from cooling tower biofilm- and planktonic phases. Other objectives included the investigation of the role of bacteria in fouling, corrosion and scaling, as well as determining population changes when operational conditions change. The main aim of her Master's study at NWU was to isolate, identify and characterize heterotrophic plate count (HPC) bacteria and other bacteria from biofilm and bulk water samples within the Potchefstroom drinking water distribution system. Objectives were to classify the tap water according to physico-chemical measurements and guideline values; to isolate and identify HPC bacteria from bulk water and biofilms of a reverse-osmosis (RO) filter system as well as an in-stream biofilm development device; to determine the diversity of isolates; to characterize bacteria in terms of (a) pathogenicity potential, (b) antibiotic resistance patterns and (c) their appearance in transmission electron micrographs; and to make use of scanning electron microscopy (SEM) to detect and study the structure of biofilms in RO filters and red-copper coupons. At the end of 2013, she received a prestigious fellowship from Southern African Biochemistry and Informatics for Natural Products (SABINA) in collaboration with Carnegie-SIG/RISE (Carnegie-Science Initiative Group/Regional Initiative in Science and Education) to pursue her doctoral studies at the University of Namibia, with a research visit to the Biochemistry Department, University of Pretoria in 2015. Her PhD research at UNAM aimed to investigate potentially pathogenic community-associated staphylococci in school children. Objectives for this study were to determine the prevalence of nasal *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS) in school children from the Mariental District, to characterize the bacteria in terms of their antibiograms and drug-resistance patterns, to screen bacterial isolates for their ability to produce enterotoxins and produce biofilms as potential virulence factors, and to assess the antimicrobial and anti-biofilm activity of crude methanolic extracts of *Aptosimum albomarginatum* (Marloth and Engl.) roots, *Albizia anthelmintica* (A. Rich Brongn.) twigs and

Dicoma schinzii (O. Hoffm.) against staphylococci (including multi-drug resistant strains) isolated from the learners. Her visit to the Biochemistry Department at the University of Pretoria entailed the screening of Namibian traditional medicinal plant extracts for anti-microbial- and anti-biofilm activity against bacteria and fungi. During her research at the Biomedical Research Laboratory at UNAM, she established the *Staphylococcus* section and co-supervised several undergraduate students on staphylococcal biofilms.

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