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An *in vitro* investigation of indigenous South African medicinal plants used to treat oral infections

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ABSTRACT

Ethnopharmacological relevance: Over a 120 South African medicinal plants are used for the treatment of oral diseases. Despite the vast collection of antimicrobial studies being done on South African plants, there is still limited research on pathogens associated with oral infections. In consultation with the available ethnobotanical literature, this study investigates the antimicrobial efficacy of some South African medicinal plants against oral pathogens.

Aim of the study: To provide a detailed account of the antimicrobial properties of selected South African medicinal plants used traditionally to treat oral infections. The effect on *Streptococcus mutans* biofilm formation and the toxicity profiles of these plants are also investigated.

Materials and methods: A total of 136 aqueous and organic extracts and six essential oils were prepared from 31 different plant species. These plant samples were screened for antimicrobial efficacy against nine oral pathogens using the micro-titre plate dilution assay. Plant extracts that were found to have noteworthy antimicrobial activity against *S. mutans* were further evaluated on the effect on *S. mutans* biofilm formation using the glass slide technique. The toxicity profiles of plant samples that were found to have noteworthy antimicrobial activity were evaluated using the brine shrimp lethality assay.

Results: The organic extract of *Cissampelos torulosa* stems displayed the lowest MIC value of 0.05 mg/mL against both *Lactobacillus* spp. This high antimicrobial activity was also observed with the organic extract of *Spirostachys africana* leaves against *Candida albicans*. In some instances, a direct relationship was found between the traditional use of the plant and the antimicrobial activity observed. For example, noteworthy activity (MIC < 1.00 mg/mL) was observed against all three *Candida* spp. when tested against *Clematis brachiata* (leaves), a plant traditionally used to treat oral thrush. *Englerophytum magalismonatanum* stems displayed notable activity against both *Streptococcus* spp. (MIC 0.83 mg/mL against *S. mutans* and MIC 0.67 mg/mL against *S. sanguis*). *Spirostachys africana* leaves displayed the greatest anti-adherent properties against *S. mutans* biofilm formation at both 24 and 48 h, reducing the biofilm by 97.56% and 86.58% respectively. The majority of plant samples tested in the brine shrimp lethality assay (BSLA) were considered safe, however, 13 plant samples were considered toxic, at a concentration of 1 mg/mL.

Conclusion: Noteworthy antimicrobial activity for plants species such as *C. brachiata* and *E. magalismonatnum* provides validation for the traditional use of these plants. *Spirostachys africana* displayed the greatest reduction of adherent *S. mutans* cells. The BSLA results revealed that the majority of the plant samples were not toxic in nature. The findings from the results favour the potential use of these plants in treating oral diseases such as dental caries, periodontal diseases and oral thrush.

1. Introduction

Oral diseases in South Africa remain a huge public health problem due to the high prevalence, severity and the cost of oral healthcare (Singh, 2011). Conventional treatment of periodontal infections includes surgery, debridements and tooth extractions making this a great

Abbreviations: Aq, aqueous extract; AIDS, acquired immune deficiency syndrome; ATCC, american type culture collection; BSLA, brine shrimp lethality assay; CFU/mL, colony forming units per milliliter; D:M, 1:1 mixture of dichloromethane: methanol; DMSO, dimethyl sulfoxide; HIV, human Immunodeficiency Virus; INT, *p*-iodonitrotetrazolium chloride; MIC, minimum inhibitory concentration; PBS, phosphate buffered saline; TSA, tryptone Soya agar; TSB, tryptone soya broth

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burden on the local healthcare system (Mohd-Dom et al., 2014). In South Africa, a review of the oral health has revealed that over 50% of children under the age of 12 suffer from dental caries and 80% of these cases go untreated (van Wyk and van Wyk, 2010).

According to the 2010 Global Burden of Diseases, Injuries and Risk Factors study, oral diseases have increased by almost 21% between 1990 and 2010. The oral diseases with the highest significant increase include severe periodontitis and untreated dental caries. Untreated dental caries was the most prevalent of all oral diseases with a global prevalence of 35% (WHO, 2016). Dental caries can be defined as the destruction of the tooth and its surrounding tissues due to plaque forming bacteria and acid by-products. These acid by-products attack the enamel and dentin of the teeth that leads to lesions and cavities (Henley-Smith et al., 2013). *Streptoccocus*, *Actinomyces* and *Lactobacillus* are the most common bacterial species having cariogenic properties (Takarada et al., 2004; Gupti, 2012). Periodontitis is characterized by the degradation of the alveolar bone and its supporting structures (Jain et al., 2008). Gingivitis and dental caries usually precede periodontal infection. It is the leading cause of tooth loss in old age and is associated with more severe systemic conditions such as cancer, diabetes and the human immunodeficiency virus (HIV) (Gupti, 2012). The most common bacteria isolated from periodontal infections include; *Fusobacterium nucleatum, Porphyromonas gingivalis*, *Prevotella intermedia* and *Actinomyces* spp. (Takarada et al., 2004; Gupti, 2012).

The *Candida* spp. are opportunistic yeasts that reside as normal microflora and manifest in immunocompromised patients (Henley-Smith et al., 2013; Kaur et al., 2014). Oropharyngeal candidiasis has become an increasingly serious public health concern and is one of the most common and earliest fungal manifestations seen in HIV and acquired immunodeficiency syndrome (AIDS) patients with a worldwide prevalence of 80–95% (Coogan et al., 2005; Kwamin et al., 2012). *Candida albicans* is the primary *Candida* spp. isolated from the oral cavity and was thought to be the predominant cause of infectious oropharyngeal candidiasis, however, *Candida* spp. such as *Candida glabrata* and *Candida krusei* have increasingly been isolated in patients suffering from oropharyngeal candidiasis (Soll, 2002; Meurman et al., 2007).

An oral biofilm is comprised of a multitude of variant micro-organisms embedded in an extra-cellular matrix allowing them to adhere and communicate with each other (Tahmourespour et al., 2010; Jakubovics and Kolenbrander, 2010; Huang et al., 2011). Oral bacteria have the ability to produce biofilms on various surfaces of the oral cavity including the solid surface of teeth and the soft surfaces of epithelial tissues (Kolenbrander et al., 2010). *Streptoccous mutans* has been discovered as the primary etiological bacteria that predominantly proliferates in the dental biofilm (Jakubovics and Kolenbrander, 2010; Kouidhi et al., 2015; Tahmourespour et al., 2010). Biofilms have been implicated as the major factor causing some severe infections.

Most plant-based antimicrobials studied have focused on planktonic micro-organisms despite the fact that micro-organisms that form in biofilms are more resistant and therefore more problematic to control and should be investigated further (Abreu et al., 2013; Varposhti et al., 2013).

Toxicology is a branch of science that deals with the adverse effects of chemical constituents on living organisms (Gowda et al., 2014). It is of great importance to investigate the toxicity profiles of medicinal plants as a major portion of the South African population still relies on medicinal plants as a first line treatment (Street et al., 2008). There is a misconception that natural remedies are safer in comparison to modern day conventional treatments, however, plants that display beneficial properties may also have cytotoxic properties (Hamidi et al., 2014). Compounds that are found in plants interact with each other or with cells that can cause toxicity (Otang et al., 2013). Acute poisoning accounts for many deaths in South Africa (Malangu and Ogunbanjo, 2009). Therefore, establishing the safety and efficacy of these plant extracts and essential oils would be beneficial.

A previous review of the South African medicinal plants and antimicrobial studies (van Vuuren, 2008; van Vuuren and Holl, 2017), has shown that despite the vast collection of antimicrobial studies done on South African plants that are used to treat a variety of ailments, there are few studies which specifically deal with South African medicinal plants and oral pathogens. Some recent studies include two separate studies on *Dodonaea viscosa,* where plant extracts have been tested against *Candida albicans*. The antifungal properties were found to be more effective than conventional mouthwashes and high concentrations of the crude extracts were found to be bactericidal against *S. mutans* (Patel and Coogan, 2008; Naidoo et al., 2012). A study involving the antimicrobial activity of eight different South African plant species used as chewing sticks was conducted by More et al. (2008). The results showed that six out of the eight plants tested exhibited minimum inhibitory concentration (MIC) values ranging from 25.0 to 0.8 mg/mL with the best activity observed for *Euclea natalensis* which inhibited the growth of both Gram-positive and Gram-negative oral pathogens. More recently, Henley-Smith et al. (2014), investigated the antimicrobial activity of the combination of South African plants with green tea extracts against oral pathogens. These combinations were successful in inhibiting the growth of the oral pathogens tested. It is surprising that so few studies have been previously conducted. Of the 132 plants that have been found relevant, only 17% of these plants have been studied before against various oral pathogens and this emphasizes the need for further research.

This study on indigenous South African medicinal plants used traditionally to treat oral infections aimed to present a detailed account of the antimicrobial properties against oral micro-organisms, its effect on biofilm formation and the toxicity profiles of plants that show antimicrobial activity.

2. Material and methods

2.1. Plant collection and processing

Plants were selected based on their availability. Different parts (depending on the traditional use) of 31 different plant species were identified and collected from the Walter Sisulu botanical gardens with permission and assistance from Mr. Andrew Hankey, specialist horticulturist. Plants not obtained from the Walter Sisulu botanical gardens were procured from Random Harvest nursery. In some instances it was not sustainable to harvest the root of the plant, therefore, other parts of the plant were collected. Voucher specimens were prepared and stored in the department of Pharmacy and Pharmacology, University of the Witwatersrand (Table 1).

2.2. Preparation and extraction of plant material

Plants were left to dry at room temperature and ground to a fine powder using the Fritsch Pulverisette grinder (Labotec). Organic extracts were prepared by immersing a known weight of the fine powdered plant material in a 1:1 mixture of dichloromethane and methanol and left in the shaker incubator (Labcon) at 37 °C for 24 h. Aqueous extracts (aq) were prepared by submerging the macerated plant material in sterile distilled water. This was kept at ambient temperature overnight. Thereafter the extracts were filtered, stored at −80 °C and lyophilized (VirtTis) (van Vuuren and Viljoen, 2006). Plant extracts were stored at room temperature in amber bottles.

Some of the plant species are aromatic in nature and therefore distilled to obtain essential oils. A known quantity of weighed fresh leaf material was subjected to hydrodistillation using a Clevenger-type apparatus. After three hours, the essential oil was collected, weighed and stored in amber bottles at 4 °C.