



Ethnopharmacological communication

Antibacterial activities of extracts from Ugandan medicinal plants used for oral care



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ABSTRACT

Ethnopharmacological relevance: Medicinal plants are widely used for treatment of oral/dental diseases in Uganda.

Aim of the study: To investigate antibacterial activities of 16 commonly used medicinal plants on microorganisms associated with periodontal diseases (PD) and dental caries (DC).

Materials and methods: Pulp juice and solvent extracts (hexane, methanol and water) from the plants were tested against *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Tannerella forsythia* associated with PD and *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus acidophilus* associated with DC. Tests were done using agar well-diffusion (pulp juice) and agar-dilution (Solvent extracts) assays. **Results:** Pulp juice from *Zanthoxylum chalybeum* and *Euclea latidens* showed activity against all the bacteria, *Zanthoxylum chalybeum* being most active. Hexane extract from aerial part of *Helichrysum odoratissimum* was most active (MIC: 0.125–0.5 mg/ml). Methanol extract from leaves of *Lantana trifolia* showed activity against all bacteria (MIC: 0.25–1 mg/ml).

Conclusion: Several of the tested plants showed antibacterial activities against bacteria associated with PD and DC, meriting further investigations.

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1. Introduction

Periodontal diseases (PD) and dental caries (DC) are common oral conditions, caused by bacterial plaque (Marcenes et al., 2013). In PD, presence of bacteria in the gingival crevice lead to inflammations that cause destruction of tissues attaching the teeth to the jawbone. Gram-negative bacteria such as *Porphyromonas gingivalis*, *Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* are associated with PD (Ximénez-Fyvie et al., 2000). DC is a destruction of dental hard tissues involving acidogenic bacteria that include *Streptococcus mutans*, *Streptococcus sobrinus* and *Lactobacillus spp.* (Takahashi and Nyvad, 2011).

The use of plants as alternative tools for oral care is an ancient custom, which remains widespread across many parts of the world

(Hyson, 2003). Extracts from some plants have been found to be effective against oral bacteria (More et al., 2008). The WHO (1987) has recommended and encouraged the use of plants as tools for oral hygiene in areas where this is customary.

In Uganda, the practice of medicinal plants for oral care is well established, but there is limited data on their antibacterial activities.

The aim of the present study was to investigate the *in vitro* antibacterial activities of fresh pulp juice and solvent extracts from 16 Ugandan medicinal plants used for oral care.

2. Material and methods

2.1. Plant materials

Plants used for traditional treatment of oral/dental diseases were selected (Table 1) and collected from different parts of Uganda during the second half of 2011. Plant parts to be used for extraction

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Table 1
Ugandan medicinal plants used for treatment of dental/oral diseases.

Family and botanical name (Voucher number) ^a	Local name/tribe	Plant part: Ethno-medical use(s) (references)	Dental/oral disease(s) treated
APOCYNACEAE <i>Carissa edulis</i> . Vahl (FO-001)	Achuga/Langi	LT: pound, add water and squeeze few drops on gum ^b	Teething syndrome
ASTERACEAE <i>Bidens pilosa</i> (FO-002)	Enyabarashana/Rukiga	LT: chew fresh ^b	Toothache
<i>Crassocephalum vitellinum</i> (FO-003)	Paroti / Kupsabiny	LT: Dry, burn and rub ash on false teeth ^b	Teething syndrome
<i>Helichrysum odoratissimum</i> (FO-004)	Chebushie / Kupsabiny	LT: Dry, burn and rub ash on false teeth ^b	Teething syndrome
<i>Vernonia amygdalina</i> (FO-005)	Okello-okello/Langi	ST: Brush teeth, twigs chewed fresh ^{b,c}	Dental caries
EBENACEAE <i>Euclea latidens</i> . Stapf (FO-006)	Amuru-dyek/Langi	RT: Use to brush teeth ^b	Dental caries
CUCURBITACEAE <i>Momordica foetida</i> K.Schumacher (FO-007)	Bomo/Langi	LT: Pound, squeeze juice onto the wound. ^b	Teething syndrome
LAMIACEAE <i>Hoslundia opposita</i> (FO-008)	Kamunye/Baganda	LT: Chew fresh ^{d,e}	Mouth wounds
<i>Ocimum gratissimum</i> (FO-009)	Omujaja/Rukiga	LT: Chew fresh ^b	Toothache
POACEAE <i>Cymbopogon citrates</i> (FO-010)	Kasubi/Baganda	LT: taken as tea; chewed fresh ^c	Bad breath, toothache
<i>Cymbopogon nardus</i> (FO-011)	Ettette/Rukiga Bad breath, dental caries.	Root: chewed; LT: Young part chewed and used for cleaning ^b	
RANUNCULACEAE <i>Clematis hirsuta</i> . Guill. & Perr. (FO-012)	Adwe/Langi	LT: pound and apply paste on painful tooth; Infusion drunk ^{b,e}	Toothache Sore throat
RUTACEAE <i>Teclea nobilis</i> . Delile (FO-013)	Nzo/Baganda	ST: Use to brush teeth ^d	Dental caries
<i>Zanthoxylum chalybeum</i> (FO-014)	Owucu/Langi Songowowo/ Pokot	RT: Use to brush; ST: Bark chewed ^e	Dental caries Toothache
SOLANACEAE <i>Solanum nigrum</i> (FO-015)	Osuga/Langi	LT, FT: crush into paste and rub on the gum ^e	Teething syndrome
VERBENACEAE <i>Lantana trifolia</i> (FO-016)	Kayuki-yuki/Baganda	ST: Use as toothbrush to clean and freshen; LT: infusion swallowed ^{d,f}	Oral Hygiene Tonsillitis

^a Voucher specimen number at Herbarium, Makerere University;

^b Mubiru et al., 1994;

^c Hirt and M'Pia, 2008;

^d Hamill et al., 2003;

^e Kokwaro, 1993;

^f Odongo et al., 2011; FT = Fruit; LT = Leaf; RT = Root; ST = Stem.

of fresh pulp juice were transported in an ice cooled box and stored at -80°C until use. Identities of the plants were confirmed by botanists at the Herbarium, Department of Botany, Makerere University, Uganda, where voucher specimens were kept.

2.2. Preparation of fresh pulp juice

Fresh pulp juice was expressed from pieces of fresh plant parts cited in Table 1 using a manual fruit juicer and tested immediately at full strength and at 50 and 25%. Dilutions were made using brain heart infusion (BHI) broth.

2.3. Preparation of solvent extracts

Preparation of solvent extracts was adapted from Matu and van Staden (2003) with modifications. Three solvents (hexane, methanol and water) were used. Hexane extract (HE) and methanol extracts (ME) were obtained by macerating 300–500 g of powdered plant material in 600–2000 ml of hexane or methanol, for 72 h and filtered using Whatman filter paper. The filtrate was concentrated using a rotary vacuum evaporator to approximately 50 ml and the resultant concentrate dried in an oven at $40\text{--}50^{\circ}\text{C}$. The extract was stored at 4°C until use.

For water extract (WE), 10–35 g of powdered plant material was heated in 200–500 ml of distilled water to boiling point for about 20 min and the extract filtered using Whatman filter paper and concentrated using a freeze dryer. The residue was stored at 4°C .

2.4. Bacterial strains and culture conditions

The bacterial strains from Culture Collection of the University of Gothenburg (CCUG) were used: *Streptococcus mutans* (CCUG 6519 T), *Streptococcus sobrinus* (CCUG 25735 T), *Lactobacillus acidophilus* (CCUG 5917 T), *Porphyromonas gingivalis* (CCUG 25226), *Aggregatibacter actinomycetemcomitans* (CCUG 56173), *Tannerella forsythia* (CCUG 21028 AT). The strains were grown and maintained as described by Sofrata et al. (2008), with the following modifications: 5% sheep blood was used instead of citrated horse blood; Columbia base agar for growing *Streptococcus mutans* and *Lactobacillus acidophilus* was not supplemented with tryptophan; *Tannerella forsythia* was grown anaerobically in chocolate agar supplemented with hemin (0.05 mg/ml) and vitamin K (0.01 mg/ml).

2.5. Antibacterial activity testing

2.5.1. Fresh pulp juice

Agar well-diffusion assay was used to test for antibacterial activities of pulp juice. *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus acidophilus* and *Aggregatibacter actinomycetemcomitans* were suspended in phosphate buffered saline (PBS). *Porphyromonas gingivalis* and *Tannerella forsythia* were suspended in peptone yeast glucose. The density of each bacterial suspension was adjusted to equal that of 0.5 McFarland standards. Each bacterial suspension was swabbed over agar plate (see Section 2.4) and a 7 mm core was removed from three positions on each plate. The wells were aseptically filled as follows: i) 0.1 ml of fresh pulp juice or dilutions; ii) 0.1 ml of doxycycline (30 µg) solution in normal saline to act as positive control; iii) 0.1 ml

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