

# Antimicrobial and phytochemical investigation of herbal suspensions used in management of oral health in Nairobi County, Kenya

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## Abstract

An increasing number of Kenyans are using natural herbal products for general and oral health care due to high cost of conventional medicine. Few of these products, however, have undergone rigorous testing, as evidenced by the limited amount of information on their safety and efficacy in the literature. This study investigated the antimicrobial and phytochemical properties of 10 suspensions based on herbal products sold in Nairobi Kenya. Phytochemical evaluation was carried out using standard methods while agar well diffusion was used to study antimicrobial work. Results reveal lack of detectable levels of phytochemicals while 50% of the investigated products lacked antimicrobial activity against test bacteria (*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* (P.a) ATCC 27853, *Proteus vulgaris* ATCC 6380, *Bacillus subtilis* ATCC 14579, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Streptococcus mutans*, *Enterococcus faecalis*, ATCC 9790 and *Lactobacillus acidophilus*). The study concludes that some products in the market may not be based on herbs as indicated in the label. Thorough investigation of antimicrobial, phytochemical work as well as toxicity should be carried out on such products. The results provide practitioners and consumers with insight into the claims of natural herbal products antimicrobial effects.

**Key words:** antimicrobial activity, herbal products, activity index, phytochemical

## 1. INTRODUCTION

The formation of biofilms in the form of plaques that harbours oral bacteria on teeth causes tooth decay and has been implicated as a cause of serious infections (Zgoda and Porter, 2001). One of the methods to manage the oral bacteria could be the use of herbal extracts which have several advantages such as unlimited availability and possibility of minimal problem of drug resistance by the oral bacteria (Pramilla *et al.*, 2012). The development of effective antibacterial agents has been accompanied by the emergence of drug-resistant organisms due to the irrational and overuse of antibiotics, failure to complete a course of treatment, genetic versatility of microbes and horizontal transfer of resistant genes among bacterial species (Pramilla *et al.*, 2012). The challenges have diminished the clinical effectiveness of antibiotics (Aibinu, 2007).

Medicinal plants can provide a wealth of antimicrobial agents, and hundreds have been investigated for biological activities. The healing property of these medicinal plants is usually linked to the presence of phytochemicals and these differ from one plant to another (Idu *et al.*, 2011). Phytochemicals are natural bioactive compounds found in plants and they are grouped in primary and secondary constituents on the basis of their functions to the plant (Krishnaiah *et al.*, 2009). Not only their presence, but also the quantity of these phytochemical constituents in a given extract determines the extent of extracts' bioactivity (Allan *et al.*, 2014). In most countries there is no universal regulatory system that guarantees the safety and activity of phytopharmaceuticals. Evidence-based efficacy of herbal medicines is still lacking in Kenya. The current study aims at validating the phytochemical components and efficacy in herbal products used for oral health management in Nairobi, Kenya.

## 2. MATERIALS AND METHODS

The current study was carried out in Nairobi County, Kenya. Herbal suspensions were randomly purchased from traditional medical practitioners in various parts of Nairobi, County. The collected samples were given sample codes HS- (herbal suspension). The samples were stored in a refrigerator at 4 °C pending analysis.

## 2.1 Phytochemistry

Phytochemical analysis for major constituents in the herbal suspension was done using standard qualitative methods as described by various authors (Houghton and Raman, 1998; Chhetri *et al.*, 2008; Ngari *et al.*, 2013a). Various groups of phytochemical components were investigated including alkaloids, glycosides, resins, phenols, amino acids, terpenoids, flavonoids and tannins that are known to contribute to antimicrobial and toxicity properties of plants.

## 2.2 Antimicrobial activity

### 2.2.1 Bacteria cultures

American type cultures collections (ATCC) from and clinical isolates from Kenyatta National hospital and department of Plant and Microbial Sciences of Kenyatta University were used. They were *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* (P.a) ATCC 27853, *Proteus vulgaris* ATCC 6380, *Bacillus subtilis* ATCC 14579, *Candida albicans* ATCC 10231, *Esherichia coli* ATCC 25922, *Streptococcus mutans*, *Enterococcus faecalis*, ATCC 9790 and *Lactobacillus acidophilus*.

### 2.2.2 Medium preparation

The Muller Hinton medium was prepared by dissolving a weighed amount in distilled water and subjected to sterilisation in an autoclave at 121 °C. About 20 ml of the molten media were poured on sterile Petri dishes and allowed to set. For *Candida albicans*, Potato dextrose agar was used.

### 2.2.3 Inoculum preparation

The bacterial cultures were maintained on sterile nutrient agar medium made of Agar (20 g), peptone (5 g), beef extracts (3 g) and NaCl (3 g) in distilled water. They were frequently subcultured and incubated at 37 °C for 72 hours. For assessment of efficacy, a fresh suspension of each culture was prepared in physiological saline solutions of 0.8 % NaCl in distilled water from a freshly grown agar slant. Each culture was streaked onto non inhibitory culture media and incubated overnight to obtain isolated colonies. Three to five well isolated colonies of the test organisms were selected from overnight growth using a sterile loop and emulsified in Mueller Hinton broth and Sabourands broth, respectively, and incubated overnight at 37 °C to give a turbidity matching 0.5 McFarland turbidity ( $1 \times 10^8$  colony forming units /ml) as described by (Ayoola *et al.*, 2008). The 0.5 McFarland turbidity was prepared by adding 0.6 ml of 1% barium chloride solution to 99.4 ml of 1 % sulphuric acid solution and mixed thoroughly. For effective comparison the turbidity was liquored in similar test tubes employed to prepare the inoculums suspension. The bacteria suspension was then compared to 0.5 McFarland's standards suspensions. Respective broth was used as the diluent to adjust turbidity of the growth to match barium chloride suspension turbidity equivalent while Sabourands dextrose broth was used for *Candida albicans*. After adjusting the turbidity of the inoculum's suspension, an aliquot 100  $\mu$ l of the inoculums was spread onto Mueller Hinton agar (MHA) plates using a sterile bent glass rod (Rojas *et al.*, 2006).

### 2.2.4 Agar well diffusion assay

For liquid samples, the modified agar well of diffusion assay (Perez *et al.*, 1997) was employed. The media were inoculated with the microorganism suspended in nutrient broth. Once the agar was solidified it was punched with 6 mm diameter wells using a sterile cork borer. The wells were filled with 10  $\mu$ l of the test samples. Negative controls were set using DMSO while Co-trimoxazole was used as positive control. Antimicrobial activity was evaluated by measuring the diameter zone of inhibition against the test organism. All the organisms that showed inhibition zone equal or greater than 7 mm were regarded as susceptible.

2.2.4. Data analysis. The zones of inhibition were expressed as mean standard deviation. Activity index was used to compare antimicrobial activity with the Co-trimoxazole.

## 3. RESULTS

### 3.1 Ingredients of herbal suspension.

Table 1 shows the ingredients indicated in the label and the claimed activities of the herbal suspensions. Results indicate that several plants such *Warbugia ugandensis*, *Mentha piperita*, *Withania sominifera*, *Rosemarinus officinallis*, *Sizygium aromaticum*, *Capsicunam annuum* and *Echenecea* species are used to prepare herbal suspension (Table I).

Table 1. Types of herbal suspensions used for oral health in Nairobi County, Kenya

| Product | Components declared on the label   | Uses claimed on the label            |
|---------|--|--------------------------------------|
| HS- 1   | <i>W. ugandensis</i> bark extracts   | Bad mouth breadth, antibacterial     |
| HS- 2   | Herbal oil   | Painkiller, antibacterial            |
| HS- 3   | <i>W. ugandensis</i> , bark extracts   | Antibacterial, heals gum disease     |
| HS- 4   | <i>W. ugandensis</i> , <i>M. piperita</i> and <i>S. aromaticum</i>                         | Antibacterial, bad mouth breadth     |
| HS- 5   | <i>Aloe vera gel</i> , <i>W. ugandensis</i> , <i>W. sominifera</i>                         | Antibacterial                        |
| HS- 6   | <i>W. ugandensis</i> bark and <i>R. officinalis</i> leaves extracts                        | Antibacterial                        |
| HS- 7   | <i>W. ugandensis</i> leaves <i>M. piperata</i> leaves and <i>S. aromaticum</i> (clove) oil | Antibacterial, heals gum disease     |
| HS- 8   | Plant sap  | Ulcers, gum bleeding                 |
| HS- 9   | <i>Aloe vera gel</i> , <i>W. sominifera</i> , <i>Echenecea</i> species                     | Antibacterial, antifungal, bad mouth |
| HS- 10  | <i>W. ugandensis</i> bark <i>C. annuum</i> fruits extracts                                 | Antibacterial antifungal             |

### 3.2. Oral conditions treated

Anti bacterial and antifungal activity was indicated in all of them. Other conditions treated include gum bleeding and management of halitosis and pain relieving activity (Table 1)

3. 3 *Phytochemicals*: The results showed that all the herbal suspensions had no detectable levels of phytochemicals under investigation.

3.4 *Antimicrobial activity*: Samples HS-1, HS-2, HS-3, HS-4 and HS-5 showed varied antimicrobial properties to all the test organisms (Table 2). The diameter zone of inhibition of product HS-1 ranged from 9-26.5 mm, while for HS-2, 7.25-22.8 mm, HS-3 (11.3-34.5 mm) and HS-5 (19.75-36.5 mm). The product HS-4 produced zone of inhibition of 33.3 and 21.3 mm on *S. mutans* and on *B. subtilis*, respectively. The same product had significant antimicrobial properties on *C. albicans*. Products HS-6, HS-7, HS-8 and HS-9 had no antimicrobial activity. The zone of inhibition of the positive control ranged from 7.8 to 33.5 mm. *S. aureas* seems to be susceptible to herbal products than the positive control (Co-trimoxazole) since all samples gave an index of more than one.

Table 2. Zones of inhibition (mm±s.e) against various microorganisms by liquid herbal suspensions used in management of oral health in Nairobi County, Kenya

| Test Organism         | Product |                |           |            |           |           |            |
|-----------------------|---------|----------------|-----------|------------|-----------|-----------|------------|
|                       | DMSO    | Co-Trimoxazole | HS-1      | HS-2       | HS-3      | HS-4      | HS-5       |
| <i>E. coli</i>        | 0       | 27.5±0.7       | 19.3±0.48 | 9.5± 1.44  | 11.8±0.35 | 33.1±0.85 | 25.3±0.25  |
| <i>S. aureus</i>      | 0       | 8.5±0.5        | 12.5±0.65 | 10.75±1.89 | 11.3±0.35 | 20.3±1.71 | 20.25±1.26 |
| <i>S. faecalis</i>    | 0       | 33.5±1.5       | 16.8±2.5  | 16.25±5.1  | 20.0±0    | 27.5±8.7  | 24.25±1.5  |
| <i>B. subtilis</i>    | 0       | 24±0.41        | 12.0±1    | 14.25±3.33 | 34.5±2.12 | 21.3±0.96 | 21.75±2.27 |
| <i>P. aeruginosa</i>  | 0       | 10± 0.0        | 9.0±0.96  | 7.25±0.43  | 13±1.06   | 19.3±1.65 | 21.5±1.71  |
| <i>P. vulgaris</i>    | 0       | 28.8±0.25      | 14.3±2.63 | 9.37±1.11  | 18.5±0.71 | 26.5±1.91 | 19.75±0.96 |
| <i>S. mutans</i>      | 0       | 7.8±0.25       | 26.5±1.29 | 11.6±1.76  | 31.5±0.71 | 33.3±1.26 | 36.5±1     |
| <i>C. albicans</i>    | 0       | 11.3±0.5       | 19.0±1.12 | 22.8±4.2   | 20.5±0.7  | 25±1.41   | 24±1.41    |
| <i>L. acidophilus</i> | 0       | 22.8±0.8       | 16.0±2.83 | 14.8±0.8   | 21.0±0    | 22.8±1.26 | 20±1.41    |

3.5 Activity index: Sample HS-4 (Table 3) produced the best activity index of 1.20 against *E. coli*. The highest AI was shown by herbal suspension HS-3, HS-4 and HS-5 against *S. mutans* (Table 3)

Table.3 Activity index of herbal pastes and suspensions used in management of oral health in Nairobi County, Kenya

| Extract        | <i>E. coli</i> | <i>S. aureus</i> | <i>E. faecalis</i> | <i>B. subtilis</i> | <i>P. vulgaris</i> | <i>S. mutans</i> | <i>C. albicans</i> | <i>L. acidophilus</i> |
|----------------|----------------|------------------|--------------------|--------------------|--------------------|------------------|--------------------|-----------------------|
| Co-Trimoxazole | 1              | 1                | 1                  | 1                  | 1                  | 1                | 1                  | 1                     |
| HS-1           | 0.7            | 1.47             | 0.5                | 0.5                | 0.5                | 3.4              | 1.68               | 0.7                   |
| HS-2           | 0.35           | 1.26             | 0.48               | 0.59               | 0.73               | 1.48             | 2                  | 0.64                  |
| HS-3           | 0.43           | 1.33             | 0.6                | 1.44               | 0.64               | 4.04             | 1.81               | 0.92                  |
| HS-4           | 1.2            | 2.39             | 0.82               | 0.89               | 0.92               | 4.27             | 0                  | 1                     |
| HS-5           | 0.92           | 2.38             | 0.72               | 0.91               | 0.69               | 4.68             | 2.12               | 0.88                  |

## 4. DISCUSSION

### 4.1 Phytochemistry

Biological activity is attributed to the presence of various secondary metabolites in plants. The lack of detectable phytochemicals in suspensions could be attributed to very low concentrations of phytochemicals that could not be detected by standard laboratory methods. Using the same method in our laboratory several phytochemicals were detected in herbal powders and chewing sticks (Ngari *et al.*, 2013a). We suspect mineral adulterants might have been used (Ngari *et al.*, 2013b) and that the herbal claims in the products' label is for marketing purposes. The findings agree with earlier work in Nigeria where evaluation of two herbal products revealed lack of phytochemicals (Okunlola *et al.*, 2007). Lack of key secondary metabolites in the liquids questions their herbal basis.

#### 4.2 Antimicrobial activity

The problem of antibiotic resistance has necessitated the need for a continued search for new antimicrobial compounds (Sibanda and Okah, 2007) and plants can be a good alternative (Pretorius *et al.*, 2003). The findings of the current work reveal antimicrobial activity in some of the herbal products. The reasonable zones of inhibition displayed by some products can be attributed to stated ingredients on the label. The antimicrobial properties of *Warbugia ugandensis* have been reported (Wube *et al.*, 2005). The antibacterial activity can be attributed to the many phytochemicals found in *W. ugandensis*.

Product HS-4 showed significant antimicrobial activity against *S. mutans* and *C.albicans* which are comparable to positive control. This is probably due to presence of *S. aromaticum* oil which has been shown to be effective against *C.albicans* (Ayoola *et al.*, 2008). The oil contains eugenol, eugenol acetate, caryo-phyllene as the major constituents. Eugenol and caryo-phyllene are known to possess antibacterial and antifungal properties. Hence the antibacterial and anti-fungal properties demonstrated by the clove oil extract can be attributed to the compounds identified (Ayoola *et al.*, 2008). Clove is also used as anodyne for dental emergencies (Prashar *et al.*, 2006). The use of tissues of *Capsicum* species in oral herbal formulations corroborates with findings by Iwu, (1993) where the extracts of the fruit are employed in various formulations like mouth gurgle to manage laryngitis.

The antimicrobial properties of *Mentha piperita* (peppermint) plant against *E. coli*, *S. aureas* and *C. albicans* has been attributed to presence of flavanoids and tannins (Pramilla *et al.*, 2012).

Presence of antimicrobial activity in some of herbal products with no detectable phytochemicals in them raises issues of herbal basis of these products. However, studies have demonstrated that antimicrobial properties of natural products can be enhanced by the addition of metal ions (Sivasankaran and Selwin, 2008). An investigation of elemental profiles carried out by Ngari *et al.* (2013b) revealed presence of various levels of minerals in herbal products. The elements could also be contributing to observed antimicrobial properties.

The current findings agree with a study evaluating antimicrobial activity of 14 commercially dentifrices containing herbal products where some products exhibited no antimicrobial activity (Lee *et al.*, 2005). The ability of the herbal extracts to inhibit the growth of the test organisms indicates lack of antimicrobial resistance of these organisms to active ingredients in the extracts. This significance cannot be over emphasized with the recent trend of high percentage of multidrug resistance to the present day antibiotics (Komolafe and Adegoke, 2008). Lack of antimicrobial properties in some products confirms the latest concern of Pharmacy and Poisons Board of Kenya on the efficacy of herbal materials in the market.

Toxicity and possible adulterants needs to be investigated before the effective products are incorporated fully in the primary oral health care systems. Additional studies are needed to determine anti plaque, anticaries and antimycotic properties of the herbal materials used in oral health.

#### CONCLUSION AND RECOMMENDATION

From the results of this study, phytochemicals in all the liquid herbal suspensions used in management of oral conditions were below detectable levels or absent. Some herbal suspensions used in Nairobi have antimicrobial properties while some had no antimicrobial activity. This implies that some herbal products in the market are not effective as advertised while some may be fake. The study recommends evaluation of chemical composition of efficacious products with undetectable levels of phytochemical constituents.

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