

Phytochemical and Antibacterial Potential of *Vernonia Adoensis* Stem Bark to Curb Cariogenic Microorganisms

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ABSTRACT

Background: Oral diseases are one of the most common diseases of humankind, which have received little attention globally. Weak health care systems, high cost of conventional oral hygiene agents and drug resistance are the major confounding factors in the fight against oral infections. Plants offer alternative arsenal of cheap and safe agents in enhancing oral health. Additionally plants present a blend of compounds which work concertedly against the microbes. This mechanisms of action provides negligible chances of drug resistance. This study was undertaken to assess the antibacterial activity of *Vernonia adoensis* against selected common oral microorganisms.

Objectives: To test the phytochemicals present and the antibacterial activity of methanol and acetone extracts of *Vernonia adoensis* stem bark against selected cariogenic microorganisms.

Methodology: The plant samples were extracted using maceration method using methanol and acetone as solvents. Phytochemicals screening was done using standard procedures with minor adjustments and antibacterial activity was done using disc diffusion method.

Results: The plant extracts were found to inhibit the growth of all selected cariogenic bacteria, except *Streptococcus pyogenes* which was found to be resistant against the acetone extract. All phytochemicals tested in the plant stem bark were present.

Discussion: The effectiveness of the plant against cariogenic bacteria could be attributed to the presence of various phytochemicals found in the plant. The present study demonstrates that the stem bark of *Vernonia adoensis* has the potential to prevent orally infectious diseases caused by the selected cariogenic microorganisms.

Keywords: Antibacterial, oral, health, hygiene, *Vernonia*, tooth decay, plants.

INTRODUCTION

Oral diseases are one of the most common diseases of humankind, which have received little attention globally. Weak health care systems, high cost of conventional oral hygiene agents and drug resistance are the major confounding factors in the fight against oral infections. Plants offer alternative arsenal of cheap and safe agents in enhancing oral health. Additionally, plants present a blend of compounds which work concertedly against microbes. This mechanisms of action provides negligible chances of drug resistance.

The World Health Organization (WHO) defines oral health as a state of being free from mouth and facial pain, oral and throat cancer, oral infection and sores, periodontal disease, tooth decay, tooth loss and other disorders and diseases that limit an individual's biting capacity, chewing, smiling, speaking and psychosocial wellbeing¹.

The mouth harbours adverse and complex microbial community. Majorly, these microbes are not harmful to the host although they can be opportunistic in case of host membranous disruption, injury or immune suppression. The microbes can also form dental cavities therefore increasing chances of infection². Studies by WHO³ indicate that, the prevalence of dental traumatic injuries in the world is on the increase, ranging from 16-40% among 6-year-old children and 4-33% among 12-14-year-old children⁴.

Herbal remedies have a long history in the treatment against gum and tooth problems. In many traditional cultures, where there are no plastic-bristle brushes or people cannot afford them, the use of herbal "chewing sticks" is a common practice⁴.

Previous studies have shown that microorganisms in the oral cavity are causative agents of oral diseases. *Enterococcus faecalis* has been found to be the most prevalent. It is approximated that 65% of the cases in root canals of teeth with apical periodontitis are caused by *Enterococcus faecalis*. *Candida albicans* was detected in 35% of the cases of root filled teeth⁵. *Staphylococcus aureus* is the most frequent species (46.4 %), followed by *Staphylococcus epidermidis* (41.1 %) in the periodontal oral cavity^{6&7}. According to Teva pharmaceuticals USA⁸, *Streptococcus pyogenes* is associated with the upper and lower respiratory tract infection. Drug resistance of these microorganisms against commonly used antibiotics is on the increase. Approximately 44% of *Streptococcus pyogenes* strains and 74 % of *Enterococcus faecalis* as well as *Enterobacter aerogenes* have been found to be resistant to tetracycline drugs. This phenomenon requires quick intervention to search for new arsenal of antibiotics.

Botanical derivatives have demonstrated great success in the fight against infectious oral microbes. *Calendula*, *Echinacea* and lavender oils inhibit the growth of *Candida albicans*, reduce inflammation and heal sores in the mouth⁹. Infected gums have been successfully treated with goldenseal or Oregon grape (*Mahonia aquifolium*). The pharmacological activity of goldenseal and *Mahonia aquifolium* has been associated to the presence of berberine which gives them their antimicrobial effects⁹.

On the other hand, *Aloe Vera* works as a natural remedy for bad breath that is caused by bacteria and fungus in the mouth¹⁰. The study on the extract of the

miswak (*Salvadora persica*) indicated that the plant has drastic antibacterial effect on the growth of *Staphylococcus aureus*. Chewing of *Salvadora persica* twice a day on a regular basis has been said to reduce the incidence of gingivitis and dental caries¹¹.

Probiotic oral hygiene products containing nisin, a natural antimicrobial agent, have the potential of inhibiting plaque accumulation and are effective in reducing gingivitis and bad breath¹². According to the study done by Kermanshah *et al*¹³ on hydro-alcoholic extract of plant *M. longifolia*, piperitone, β – caryophyllene, 1,8 Cineol and some flavonoids like hesperidin and quercitrin were extracted from the plant, these compounds were found to have bactericidal effect against *S. mutans* and *Lactobacillus* species. *S. officinalis* inhibited *Lactobacillus* which is a clinically important cariogenic bacteria. Previous studies have also shown that, acetonc extract of *P. anisum* which has trans-anethole and β -caryophyllene has antibacterial activity. Flavonoids like epigenin and isovitexinis are effective against *Staphylococcus aureus* and its essential oil has good effect against a wide spectrum of bacteria. Polyphenol compounds from medicinal plants have anti-cariogenic effect, these compounds have been revealed to inhibit the growth of cariogenic bacteria such as *Streptococcus mutans* and *lactobacillus acidophilus* in the prevention of dental caries¹⁴.

An African chewing stick *Garcinia kola* showed antibacterial activity against dental caries clinically important *Streptococcus mutans*¹⁵. According to Mohan *et al*¹⁶, Herbal tooth pastes and conventional tooth pastes have similar antibacterial activity against cariogenic bacteria. A study published by Bairwa *et al*¹⁷ focusing on medicinal plants use in oral hygiene proved that, numerous plants are used to prepare plant based tooth pastes and

gels, Propolis a resin rich in flavonoids demonstrated significant antimicrobial activity against microorganisms such as *S. mutans*, *S. sobrinus* and *C. albicans* which are involved in oral diseases¹⁸. A study by Larsen *et al*¹⁹, showed alkaloid extract of *Sanguinaria Canadensis* possesses antibacterial effect against oral bacteria *in vitro*.

Vernonia is a genus in the family *Asteraceae* and has many species of forbs and shrubs²⁰. The plant *Vernonia adoensis* is used by Nandi and Kamba people in Kenya for oral health. Some species of *Vernonia* such as *Vernonia cruda*, *Vernonia colorata*, and *Vernonia amygdalina* have also shown antimicrobial activity. *Vernonia hymenolepis* is ethnobotanically used to treat toothache in Kenya²¹. This study was undertaken to assess the antibacterial activity of *Vernonia adoensis* against selected common oral microorganisms.

Materials and Methodology

Sample Collection and Preparation

The *Vernonia adoensis*, plant was collected in the natural forests around University of Eastern Africa, Baraton, Nandi County, Kenya. The stem bark was obtained, air dried in shade at room temperature and grounded in to fine powder using an electric laboratory mill.

Extraction procedure

Using analytical beam balance, 94 grams of grounded bark sample was weighed and submerged into a 400ml conical flask with 90% acetone²². The 400ml conical flask with the mixture was placed on the shaker and agitated for thorough mixing for 24 hours. The sample mixture was filtered using Buchner funnel with whatman no. 1 filter paper through the help of a vacuum pump. The filtrate was concentrated to dryness using a rotavapor machine and vacuum pump at 40°C, and the

crude extract was stored at 4°C until use. The same procedure was repeated with new sample and 90% methanol instead of acetone.

Qualitative phytochemical analysis

The phytochemical analysis on the extracts was done using standard procedures to test for tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, steroids and phenols with minor adjustments²³.

Media preparation

The plates and test tubes used in this study were sterilized in an autoclave at 121°C for 15 minutes. Microorganisms were first grown on nutrient agar media to confirm their viability. The nutrient agar media was prepared according to the manufacturer's instructions and transferred into sterilized plates and test tubes respectively. Approximately 20ml of the prepared media was poured in to the sterilized plates and 5ml of the broth was transferred in to sterile test tubes. The plates were then incubated at 37°C for 24 hours to confirm their sterility. The transfer of the media to the plates and test tubes was done under a sterile biosafety cabinet hood.

Preparation of the Extracts Concentrations and Antibiotic

Extracts stock solutions were prepared by dissolving 100mg of the concentrated plant extracts in 1ml of dimethyl sulfoxide (DMSO) and the positive control was made by dissolving 100 mg of penicillin in 1 ml of sterile distilled water, while 100% DMSO served as the negative control.

Anti-bacterial screening

Nutrient agar and broth were prepared according to the manufacturer's instructions. The media and the broth were sterilized in an autoclave at 121°C for 15

minutes. The plates were later flamed on the surface using a Bunsen burner (non luminous) flame to remove air bubbles. The plates and all the other equipment used in the experiment were transferred in to a biosafety cabinet hood. The germicidal lamp of the hood was put on for 30minutes to sterilize the surface of the plates and all the other equipment. The bacterial suspension from the test tubes was smeared on the media by the use of sterilised cotton swab. Sterile Kirby-Bauer discs were impregnated with 0.1ml of the 100mg/ml of the extracts, with 0.1ml of 100mg/ml of penicillin (positive control) and 0.1ml of 100% DMSO (negative control). The discs were aseptically placed on the prepared sterilised solid medium of nutrient agar in the plate in triplets for the plant extracts and one disc for the positive and one as negative control. The plates were then labelled on the underside and incubated at 37°C for 24 hours²⁴.

Data analysis

The zones of inhibition were measured in millimetres with the aid of a meter rule. The mean and standard error of methanol and acetone extracts and penicillin (positive control) against cariogenic bacteria were compared to check for significance in their differences ($p \geq 0.05$) using one-way ANOVA analysis of variance by SPSS software version 20.0.

Results and Discussion

The adoption of modern approaches on oral hygiene using synthetic formulations has led to diminished use of ethnobotanical knowledge on oral health. According to Devi *et al*²⁵, mostly used herbal extracts from herbal plants have demonstrated antiplaque and antibacterial effect against infections caused by oral bacteria. The use of some plants has been found to be highly effective to expedite the prevention and curing of gum disease, oral mucosal diseases

and tooth decay²⁶. The beneficial medicinal effects of plant materials including the antibacterial activity typically resulting from the secondary metabolites present in plant remains to be one of the untapped potential in the fight against oral infections. The action of herbs against infections is not normally attributable to a single compound but a combination of the metabolites. This makes them better candidates in the fight against drug resistance.

The results obtained from phytochemical screening of *Vernonia adoensis* stem bark extracts, important pharmacological compounds viz phenols, saponins, tannins and flavonoids were found to be present in the plant. The methanol extract and acetone extract exhibited comparable antibacterial zones of inhibition against selected clinically important cariogenic bacteria. The acetone extract exhibited large zones of inhibition than methanol extract despite its inability to inhibit the growth of *Streptococcus pyogenes*. In methanol extract, *Enterobacter aerogenes* (13.00± 0.577mm) had the largest zones of inhibition followed by *Streptococcus pyogenes* (11.00± 0.577mm), *Staphylococcus epidermidis* (9.67±0.333mm) and *Enterococcus faecalis* (9.00±0.577mm) respectively. In the zones of inhibition obtained from acetone extract, *Enterobacter aerogenes* (16.00±0.577mm) had the largest zones of inhibition followed by *Staphylococcus epidermidis* (11.33±0.882mm), *Enterococcus faecalis* (10.00±0.577mm) and *Streptococcus pyogenes* (0.00±0.000) which was resistant to acetone extract respectively. The antibacterial activity of the plant extract could be attributed to the present phytochemicals. According to the study by Cyriac *et al*²⁶, tannins present in the plant extracts have an astringent effect on the mucous membrane and they form a layer over enamel, thus providing protection

against dental caries while alkaloids promote antibacterial activity of plant against oral bacteria. According to the study by Ngule *et al*²⁷, the plants flavonoids were associated with the inhibition of low-density lipoproteins oxidation, thus preventing free radicals that may cause periodontal diseases and stress on cells leading to oral tumour development in the long run. Plants contain active constituents such as tannin, flavonoids, saponins and alkaloids which have antimicrobial activity against some common pathogenic strains that pose great threat to oral health²⁸. Plants have been found to be safer and effective in curing oral diseases since the use of fluoride based toothpaste has been associated with adverse oral health effects and use of hard brush causes abrasion to the gum. Plants have a long heritage in dental treatment, improvement of oral hygiene, prevention of gum disease, tooth decay and periodontitis²⁹. It is therefore important to explore new arsenal of therapeutic agents. Plants have shown to be a potential source of the next generation of antibiotics against cariogenic bacteria¹⁷.

Conclusion

In conclusion, the inhibitory effect against cariogenic bacteria exhibited by *Vernonia adoensis* stem bark indicate the presence of effective active compounds such as tannins, flavonoids, and phenols which aid in controlling oral infectious diseases. However, further research needs to be done to investigate important bioactive compounds in the plant. Test for safety profiles need to be done. Synergistic action of the phytochemicals against these bacteria in the *in vitro* and *in vivo* should be ascertained. The phytochemicals isolated from the plant will act as makers in the formulation of herbal oral hygiene remedies for *Vernonia adoensis*. The bioactive compounds extracted will also be used as

indicators in the standardization of the plants medicinal use in the fight against oral infections.

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Conflict of Interest declaration

The authors declare no conflict of interest.

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Table 1. Zones of Inhibition of *Vernonia adoensis* Stem Bark Extracts of Methanol and Acetone against selected clinically important cariogenic bacteria

Cariogenic Bacteria	Methanol extract inhibition zone mean \pm S.E(mm)	Acetone extract inhibition zone mean \pm S.E(mm)	Penicillin inhibition zones mean \pm S.E(mm)	DMSO mean \pm S.E(mm)
<i>Enterococcus faecalis</i>	9.00 \pm 0.577	10.00 \pm 0.577	38.00 \pm 0.577	0.00 \pm 0.000
<i>Streptococcus pyogenes</i>	11.00 \pm 0.577	0.00 \pm 0.000	43.33 \pm 0.882	0.00 \pm 0.000
<i>Staphylococcus epidermidis</i>	9.67 \pm 0.333	11.33 \pm 0.882	19.33 \pm 0.333	0.00 \pm 0.000
<i>Enterobacter aerogenes</i>	13.00 \pm 0.577	16.00 \pm 0.577	36.33 \pm 0.882	0.00 \pm 0.000

Key: S.E= Standard Error

Table 2: Turkey's pairwise comparison of the zones of inhibition caused by the plants (acetone) extract and penicillin against selected clinically important cariogenic bacteria

Comparison	p-value($p \geq 0.05$)	Sig.
<i>E. faecalis</i> vs <i>S. pyogenes</i>	0.000	S
<i>E. faecalis</i> vs <i>S. epidermidis</i>	0.949	NS
<i>E. faecalis</i> vs <i>E. aerogenes</i>	0.000	S
<i>E. faecalis</i> vs <i>E. Faecalis</i> control	0.000	S
<i>S. pyogenes</i> vs <i>S. epidermidis</i>	0.000	S
<i>S. pyogenes</i> vs <i>E. aerogenes</i>	0.000	S
<i>S. pyogenes</i> vs <i>S. Pyogenes</i> control	0.000	S
<i>S. epidermidis</i> vs <i>E. aerogenes</i>	0.002	S
<i>S. epidermidis</i> vs <i>S. epidermidis</i> control	0.000	S
<i>E. aerogenes</i> vs <i>E. aerogenes</i> control	0.000	S

Key: S= Significant NS= Not Significant

Table 3: Turkey's Pairwise Comparison of the Zones of Inhibition Caused by the Plant (Methanol) Extract and Penicillin against clinically important cariogenic bacteria

Comparison	p- values	Sig
<i>E. faecalis</i> vs <i>S. pyogenes</i>	0.505	NS
<i>E. faecalis</i> vs <i>S. epidermidis</i>	1.000	NS
<i>E. faecalis</i> vs <i>E. aerogenes</i>	0.005	S
<i>E. faecalis</i> vs <i>E. faecalis</i> control	0.000	S
<i>S. pyogenes</i> vs <i>S. epidermidis</i>	0.917	NS
<i>S. pyogenes</i> vs <i>E. aerogenes</i>	0.505	NS
<i>S. pyogenes</i> vs <i>S. pyogenes</i> control	0.000	S
<i>S. epidermidis</i> vs <i>E. aerogenes</i>	0.013	S
<i>S. epidermidis</i> vs <i>S. epidermidis</i> control	0.000	S
<i>E. aerogenes</i> vs <i>E. aerogenes</i> control	0.000	S

Key: S= Significant NS= Not Significant