

***Vernoa hildebrandtii*, *Acacia stuhlmannii* and *Moringa oleifera* Leaf and Root-bark Extracts Exhibit Antimicrobial Effects on *Escherichia coli* and *Staphylococcus aureus* Bacteria**

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Abstract

Infectious diseases are the main causes of morbidity and mortality worldwide. There is increasing concern of indiscriminate use of antibiotics and incidences of multiple antibiotic resistances in human pathogens. The potential of higher plants as source of new drug leads has been demonstrated but is still under explored. In Africa and most developing countries, traditional medicine still forms the backbone of rural medicinal practice. Although a number of American and Asian countries medicinal herbs have been evaluated scientifically and their medicinal properties demonstrated. In Africa, attempts to evaluate medicinal plants in relation to their biological activities and medicinal usefulness are limited. The emergence of antibiotic resistance has led to increased use of herbal medicine as an alternative to combat various ailments. This study aimed at determining the antimicrobial activity of *Vernoa hildebrandtii*, *Acacia stuhlmannii* and *Moringa oleifera* leafy and root bark extracts using disc diffusion technique. Crude extracts were obtained from dried powder by single solvent maceration with ethanol and water. Bioassays were used to evaluate the bioactivity of the extracts against *Escherichia coli* and *Staphylococcus aureus*. Antimicrobial activity was determined by measuring the zones of growth inhibition in mm. *Moringa oleifera* root water extract was the most potent fraction with bioactivity range of between 6-32 mm followed by *Acacia stuhlmannii* root bark water extracts with bioactivity range between 12-31 mm and *Moringa oleifera* root bark with bioactivity range of between 6-29 mm. *Vernoa hildebrandtii* leaf alcohol and *Acacia stuhlmannii* Taub root bark- alcohol extract had bioactivity range of between 9-28 and 5-28, respectively. However, *Acacia stuhlmannii* leaf extracts did not show any antimicrobial activities. The antimicrobial effects of the plant extracts were dose dependent. These findings validate what have been known about *Moringa oleifera*. They also demonstrate potential biochemical agents in *Vernoa hildebrandtii* and *Acacia stuhlmannii* extracts in the management of gram positive and gram negative bacteria.

Key Words: Antimicrobial Effects, *Vernoa hildebrandtii*, *Acacia stuhlmannii*, *Moringa oleifera*

Introduction

Antibiotic and antimicrobial agents have been used to treat infectious diseases for the last 70 years (Anwar et al., 2000). Since 1940s, antibiotics were reported to reduce the burden that infectious diseases pose to human health (Runyoro et al., 2006). However, the long term use of these drugs has given a chance to microorganisms to adapt and make the drugs less effective. The emergence of multidrug resistant pathogens such as *Escherichia*

coli, *Klebsiella pneumoniae* and *Candida albicans* has been a major challenge in the management of these diseases caused by these pathogens (Diallo et al., 2002).

Vernoa hildebrandtii is a tropical plant found in the coastal counties of Kenya; Tana-River, Kilifi and Kwale counties and belong to the family Compositae. This plant grows in the humid tropics or hot dry land with average height that ranges from 0.5m to 5m. It is a woody creeping plant with

white and purple floral parts. It is locally known as Mrusapung in Swahili, (Chi)watsa in Digo, Mlajakoma in Giriama and Mlalapiri in Chonyi (Pakia et al., 2003). For a long time, the Mijikenda community have used this plant to treat diarrhea, stomach ache, vomiting, and other ailments (Greenway et al., 1969a). *Acacia stuhlmannii* is a member of the family Mimosaceae. It is a multipurpose nitrogen fixing tree legume which occurs from sea level to over 2000 m (Adnan et al., 2018). It withstands extreme temperatures of above 50°C and air dryness but sensitive to frost when young (Bargali et al., 2009). It is widely spread in subtropical and tropical Africa from Egypt to Mauritania southwards to South Africa and in Asia (Runyoro, et al., 2006; Jigam et al., 2010). In Kenya, the plant is found in Tsavo East and the counties of Kwale and Kilifi (Greenway et al., 1969).

Moringa oleifera is one of the best known medicinal plants. It belongs to the family Moringaceae (Jigam et al., 2010). It is also known as horse-radish tree, drumstick. Another vernacular name is *Mzungi* as it is well known among the Mijikenda communities of Kilifi in coastal Kenya. It is a small, fast growing, evergreen, or deciduous tree that usually grows up to 10 or 12m in height. The species is native to Kilifi and also grows in tropical and subtropical regions. It is distributed among sub-Saharan Africa and India. It has its beneficial role in cancer, diabetes, liver treatment, ulcer and various other diseases. Similarly, it has applications as an antioxidant, immunosuppressant, cytoprotective, and gastro protective (Farooq et al., 2012)

In Africa and most developing countries, traditional medicine still form the backbone of rural medicinal practice (Toda et al., 2016). Medicinal herbs are used extensively for various ailments in these countries. Although a number of American and Asian countries' medicinal herbs have been evaluated scientifically and their medicinal properties known, up to now there are no serious attempts to evaluate African medicinal plants in a collection of their biological activities and medicinal usefulness (Runyoro et al., 2006). With the emergence of antibiotic resistance, herbal medicine has been the target alternative to combat various ailments. This study aimed at determining the antimicrobial activity of *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* leaf and root-bark extracts. This research will ignite the advantage of

the exciting medicinal plants in and their application to modern medicine and improvement of the usage of these herbs which are under-utilized in Kenya and other parts of sub-Saharan Africa.

Materials and Methods

Plant Materials

Acacia stuhlmannii leaves, and root barks, and *Moringa* leaves were collected from Kwale County in Msambweni area located at 4.57°S longitude and 39.28° E latitude and at an altitude of 2.2 m above the sea level, while *V. hildebrandtii* leaves and root barks were collected from Kilifi County in Chonyi area located at 3.78°S longitude and 39.7° E latitude and at an altitude of 2.7 m above the sea level.

Test Microorganisms

Escherichia coli and *Staphylococcus aureus* were obtained from the American Type Culture Collection (ATCC) and propagated under appropriate conditions. The cultures were maintained on nutrient agar slants at 4°C and sub-cultured before use.

Preparation of Plant Extracts

The plant samples were washed with sterile water, and kept under a shade till drying. Extraction from leaves and root barks were carried out by simple maceration process as described by Savithramma et al., (2011) with some adjustments. The specimens were grounded into coarse powder then suspended in 75% ethanol and water for 3 to 7 days at 37°C in extraction bottles. Filtration of extract was done twice using Whatman-41 filter paper. Concentrations of crude extracts were done at a temperature range of 40°C-50°C using R209 rotary evaporator. The ethanol and water were completely evaporated by rotary evaporation to obtain the extracts. The extracts were stored at 4°C till use.

Preparation of Samples

Each extract was dissolved serially in 1 ml of 10%v/v DMSO to make concentrations covering 15, 20, 30, 40 and 50 mg. Using DMSO, adjustments were made such that 15 µl of each was delivered on to sets of filter paper disks, each receiving approximately 15µl of solution.

Preparation of Media for Bacteria

Nutrient broth medium was prepared by dissolving 0.4 g/50 ml of distilled water for the growth of bacteria inoculums; pH was adjusted at 7.0 and autoclaved. Nutrient agar medium was prepared by dissolving 2.3g/100 ml of distilled water at pH 7.0 then autoclaved at 121°C.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility was determined by agar well diffusion and disc methods (Kabbashi et al., 2016). Muller Hinton Agar (MHA) medium was prepared and sterilized by autoclaving at 121°C for 15 min as described by Kabbashi, (2016). The media was poured into sterile Petri plates and allowed to solidify.

Escherichia coli and *Staphylococcus aureus* were inoculated by spreading onto the surface of the MHA media by using sterile cotton swabs. Sterile discs impregnated with plant extract were seeded on the plates and incubated at 37°C for 24 hrs. Antimicrobial susceptibility test was done in duplicate using Gentamicin, Ciprofloxacin, Ceftriazone and Nitrofurantone as positive controls and 10%v/v DMSO as negative control based on the CLSI susceptibility protocol. Zones of growth inhibition in mm were evaluated after 24 hrs of incubation. The lowest concentration of the crude extract that shows zones of growth inhibition is the minimum inhibitory concentration. Antimicrobial activity was evaluated by measuring the zones of growth inhibition against the tested microorganisms.

Data Analysis

Antimicrobial activity was an average of zones of growth inhibition in mm recorded from the duplicates. The mean diameter score was analyzed as a fraction of the extract concentration and solvent type. Statistical analysis was done using 1-Way ANOVA in Minitab software. P values ≤ 0.05 were considered significant.

Results

Leaf Water Extracts of *Vernoa hildebrandtii*, *Acacia stuhlmannii* and *Moringa oleifera* Inhibit Bacteria Growth

Here we explored the potential of water leaf extracts of *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* to inhibit the growth of bacteria. Leaves

were grinded and dissolved in water for 7 days at 37°C.

The solvent was evaporated and extracts applied to *E. coli* and *S. aureus*. Results showed that the leaf extracts of *V. hildebrandtii* and *M. oleifera* inhibit the growth of *E. coli* and *S. aureus* bacteria in a dose dependent fashion (Fig. 1a & b). At the same time, *A. stuhlmannii* did not show any antimicrobial activity trend against *E. coli* and *S. aureus* ($p = 0.034$; Figs. 1a & b).

Leaf alcohol extracts has antimicrobial properties against *E. coli* and *S. aureus*

The potential of alcohol leaf extracts of *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* to inhibit bacteria growth was explored. Leaves were grinded and dissolved in alcohol for 7 days at 37°C. The solvents were evaporated and extracts applied. Screening antimicrobial activity revealed inhibitory activity by *V. hildebrandtii* and *M. oleifera* against *E. coli* and *S. aureus* (Fig. 2a & b). *Acacia stuhlmannii* leaf extracts did not show any antimicrobial activity trend against *E. coli* and *S. aureus* ($p < 0.01$; Fig. 2a & b).

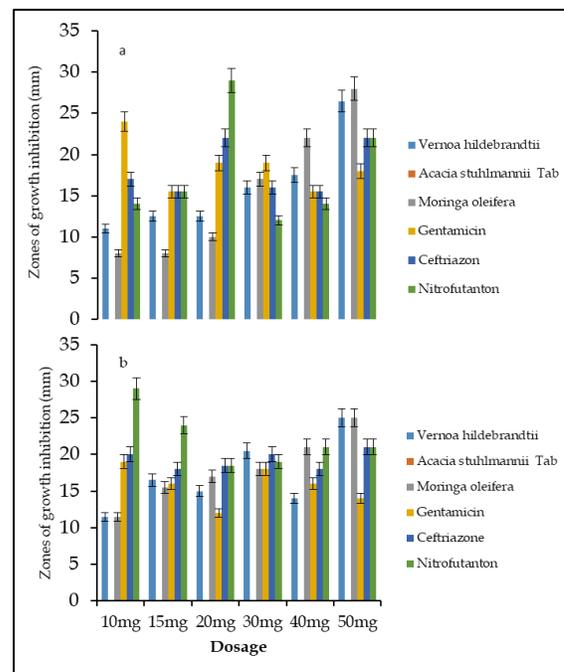


Figure 1. Antimicrobial activity of water leaf extracts against a) *E. coli* and b) *S. aureus* by disc diffusion method

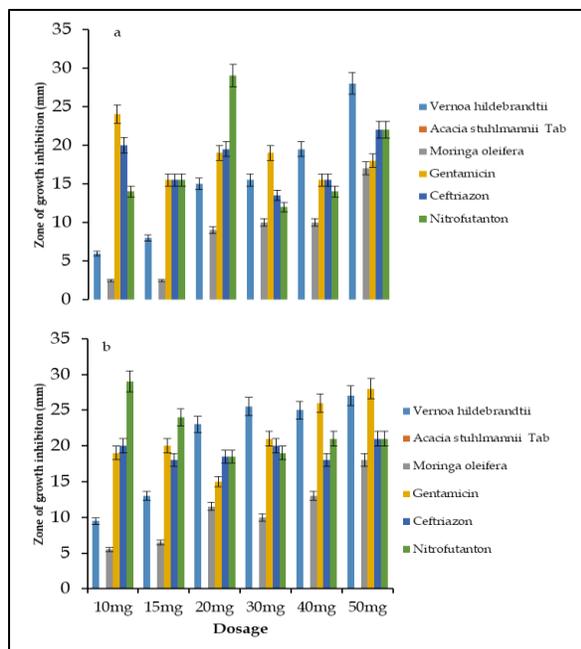


Figure 2. Antimicrobial activity of leaf alcohol extracts against a) *E. coli* and b) *S. aureas* by disc diffusion method

Root-bark Water Extracts of *Moringa oleifera* has the Highest Antimicrobial Activity

To determine antimicrobial property of water root bark extracts of *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera*, root-bark were grinded and dissolved in water for seven days at 37°C. The solvents were evaporated and extracts applied. Screening antimicrobial activity revealed inhibitory activity by *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* against *E. coli* and *S. aureas* with *M. oleifera* recorded as the highest fraction with bioactivity range of between 6-32 mm ($p < 0.01$; Fig. 3 a & b).

***Vernoa hildebrandtii* Root-bark Alcohol Extract does not have Antimicrobial Activities at Lower Concentration**

Figure 4 a & b shows a consistent increase in microbial activity as the dose increases at higher doses starting from 20 mg. There was no inhibition recorded between 10-15 mg concentration ($p = 0.670$).

Discussion

Healing properties of medicinal plants that are used to manage infectious diseases has been proven by several studies conducted to evaluate antimicrobial activities of medicinal plants (Mahesh et al., 2008; Kabbashi et al., 2016). The first step towards development of new chemotherapeutic agents is in vitro antimicrobial activity assay (Mahesh et al., 2008). When tested by the disc diffusion method, *M. oleifera* root water extracts were the most potent fraction with bioactivity range of 6-32 mm (Fig. 3a) against *E. coli* followed by *A. stuhlmannii* root-bark water extracts which had bioactivity range of 12.5-31.5 mm against *S. aureas* (Fig. 3b). *Moringa oleifera* root-bark water extract had bioactivity range of 6.5-29.0 mm (Fig. 3a). *Vernoa hildebrandtii* leaf alcohol (Fig. 2a) and *A. stuhlmannii* root-bark alcohol extract (Fig. 4a) had bioactivity range of 9.5-28 and 5.8-28, respectively. However, *A. stuhlmannii* leaf extracts using both water and alcohol against *E. coli* and *S. aureas* did not show any antimicrobial activities (Figs. 1 & 2) contrary to *Acacia aroma* which has been shown to have antimicrobial activities against some gram-negative bacteria in other studies (Mattana et al., 2010). *Vernoa hildebrandtii* root-bark extracts did not show antimicrobial activities against *E. coli* and *S. aureas* at lower doses of 10-15mg ($p = 0.670$; Figs 4a & b).

Taken together these results clearly indicate that, antimicrobial activity varies with the species of the plants, extraction solvent used and specific parts of the plant material used. Further these results showed a dose dependent antimicrobial activity of *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* against gram positive and gram negative bacteria.

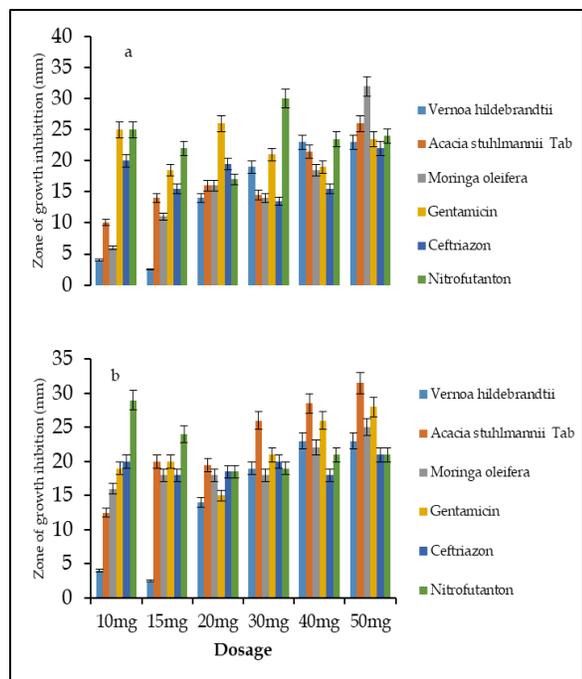


Figure 3. Antimicrobial activity of root bark water extracts against a) *E. coli* and b) *S. aureas* by disc diffusion method

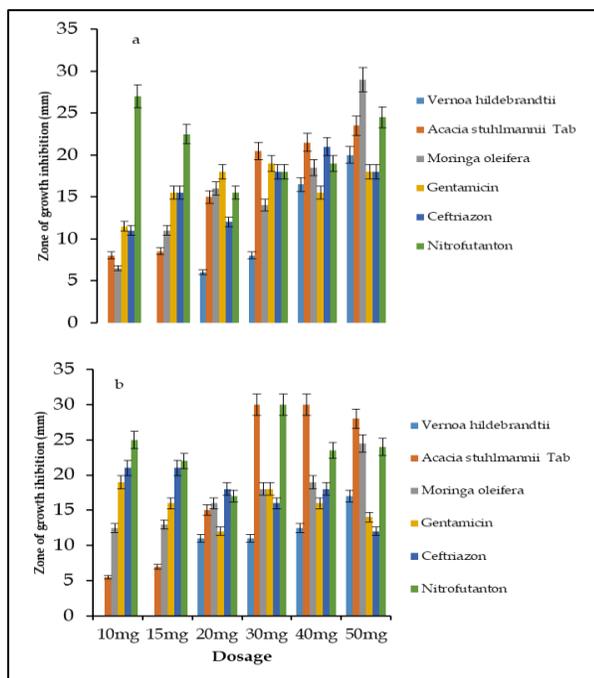


Figure 4. Antimicrobial activity of root bark- alcohol extracts against a) *E. coli* and b) *S. aureas* by disc diffusion method

Conclusion

Our results suggest that, *V. hildebrandtii*, *A. stuhlmannii* and *M. oleifera* possess compounds with antimicrobial properties which can be useful in designing new therapeutic agents. Further work is under way to elucidate the mechanisms of action.

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