In Vitro Susceptibilities of *Staphylococcus Aureus*, *Candida Albicans, Pseudomonas Aeruginosa and Escherichia Coli* to the Inner Gel of *Aloe Nuttii* Baker and *Aloe Chabaudii* Schonland Found in Zambia

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ABSTRACT

The genus *Aloe* has a long history of medicinal usage worldwide and is believed to treat various ailments. It contains bioactive compounds that work synergistically against various microbes. A comparative study of the two Zambian Aloe species, *Aloe nuttii Baker* and *Aloe chabauddii,* was done with the objectives of determining the antimicrobial activity, the minimum inhibition concentration and the susceptibility of microbes, namely; *Staphylococcus aureus*, *Candida albicans, Pseudomonas aeruginosa and Escherichia coli.*

The study was a quantitative, comparative, laboratory-based study. A Liquid-Liquid extraction method modified from Marjory Cowan, 1999 was used. The extracts were tested on microbes using the Agar Disk Diffusion Method modified from Kirby Bauer 1966 and assessed by the growth inhibition zones. The two-fold dilution method modified from Doughari, 2007 was used to find the Minimum Inhibitory Concentration.

Only the water extracts of *Aloe nuttii Baker* inhibited the growth of all the five microbes tested as indicated by the growth inhibition zones as follows: *S. aureus* 12±2mm, S.spp.10.3±*0.7mm, P. aeruginosa* 9.3±1.7mm, E. coli 10.7±1.3mm and *Candida albicans* 14±0.6mm. In contrast, *Aloe chabaudii* extracts displayed no antimicrobial activity. The MIC of *Aloe nuttii Baker* was found to be 1500mg/ml for *Candida albican*, *P. aeruginosa* and E. coli and 3000mg/ml for *S. aureus*. In contrast, *Aloe chabauddi* did not inhibit any microbial growth.

In-vitro, *Staphylococcus aureus, Candida albicans, Pseudomonas aeriginosa* and *Escherichia coli* were susceptible to the inner gel of Aloe nuttii Baker but, in contrast, showed no susceptibility to the inner gel of aloe chabaudii schonaland found in Zambia.

Keywords: In-vitro susceptibility, Aloe *nutti* baker, Aloe *chabaudii* schonaland, antimicrobial activity, minimum inhibitory concentration, Zambia.

INTRODUCTION

Aloe species are well known for their considerable medicinal properties. Over 200 biologically chemical compounds have been isolated from aloe species. The inner gel of aloe leaves has been identified as being largely responsible for the observed biological activity. Most aloe research has focused on the antimicrobial properties of the non-volatile components of the leaf gel. Aloe species are predominantly found in Africa and Eastern Europe but have been found in other parts of the world. The genus Aloe consists of over 400 species; however, only aloe vera (A. vera) A. ferrox and A. arborescens are commonly traded commercially around the globe.

Despite the absence of many valid research outputs, A. vera, for instance, is claimed to relieve constipation, gastrointestinal disorders, diabetes and immune deficiencies[1].

Aloe species have very high water contents, often as high as 99-99.5%, while the remaining 0.5-1.0% is made up of more than 75 compounds that include water and fat-soluble vitamins, minerals, enzymes, polysaccharides, phenolic compounds and organic acids [2,3]. Among the aloe species, A. vera is one of the most extensively studied. This species has been used for centuries for its reputed medicinal properties, but despite having over 75 biologically active compounds identified, none of these has been assigned to the observed curative effects [4]. The therapeutic effects of aloe have, however, been ascribed to the leaf polysaccharides [5]. There is, however, a general agreement that the biological activities of aloe extracts must be attributed more to the synergy of multiple compounds rather than any single constituent [6]. Several secondary metabolites have been identified in aloe gel, including anthraquinones and tricyclic aromatic quinines, which make up the main compounds present. Aloe emodin is the major anthraquinone derivative isolated. Additionally, aloesin and aloin are the other significant secondary metabolites found in A. vera gel.

These metabolites have been reported to possess powerful anti-inflammatory, lipid-lowering and antioxidant properties [7,8]. Aloe leaf extracts have been used for wound healing in many cultures.

Although the use was previously anecdotal, it has been demonstrated in recent years via in-vitro studies that the extracts stimulate cellular proliferation, resulting in observably faster wound healing. This may be due to direct effects on wound contraction and stimulation of collagen synthesis by mannose-6-phosphate phosphate present in the leaf gel. Other beneficial effects arise from fibroblast proliferation, which produces hyaluronic acid and hydroxyproline, which are important in remodelling the extracellular matrices during wound healing. Alginate/aloe vera hydrogel films for wound healing applications have been prepared by Pereira, Mendes and Bártolo for wound healing and other biomedical

applications like drug delivery [9–14]. Aloe gel wound dressings have also shown superior efficacy in wound healing compared to 1% silver sulfadiazine. In vivo studies in rats have shown that the aloe polysaccharides increase both the production of matrix mellatopeptidase (MMP)-3 and the expression of inhibitor-2 gene during the wound repair process with an overall beneficial effect on wound healing[15,16].

The antimicrobial properties of A. vera have been reported in recent years. For instance, the leaf gel has shown potent activity against the Candida species C. paraprilosis, C. krusei, and C. albicans. The aloe leaf anthraquinones have structural similarity to the antibiotic tetracycline. Anthraquinones have been demonstrated to act much like tetracycline's in inhibiting the bacterial ribosomal A site, which is the entry point for aminoacylated tRNA, resulting in the curtailment of protein synthesis. Bacterial colonies are unable to grow in culture media containing aloe extract, and it has been established that both Gram-negative and Gram-negative bacteria are susceptible to the leaf gel [4,17,18]. The aloe polysaccharides stimulate the activity of phagocytic leucocytes that directly kill bacteria. A. vera also contains a hydroxylated phenol known as pyrocatechol, that is toxic to a number of microorganisms [19–21]. Tan, Li, and Xiang have demonstrated the activity of A. vera leaf gel against both susceptible and resistant strains of Helicobacter Pylori. The study further suggests the use of the leaf gel concomitantly with antibiotics as a new therapeutic option for H. pylori infection [22]. The anthraquinone derivatives, aloe-emodin, emodin, and chrysophanol, have antiviral activity against influenza A, while consumption of the leaf gel of A. vera increases CD4 count, as shown in preliminary studies. Herpes simplex virus type 2 is also susceptible to the crude extracts of A. vera gel [23–25].

MATERIALS AND METHODS

Preparation of Aloe Gel

Fresh leaves (1 kg each) of *Aloe nuttii* Baker and *Aloe chabaudii* Schonland were collected from Mutinondo in Mpika District, Muchinga Province, Zambia, in March 2015. The plants were identified in the herbarium of the Department of Biological Sciences at the University of Zambia, and specimens were deposited. The leaf gel was harvested from the fresh green leaves, homogenised, then dried at 35^{o}C for 2 hours in an oven and weighed.

Preparation of Plant Extracts

The powder of each *Aloe* species (100g each) was transferred into separate amber bottles containing 50ml each, of methanol, ethanol and distilled water and allowed to stand for 24 hours at 25 ^{o}C. The solvents were filtered through Whatman No. 1 filter paper into 6 separate collection conical flasks. The solvent was evaporated at a low temperature (40^{o}C) and reduced pressure in a rotavapor. Between 60 to 63g of dried extracts were obtained after evaporation. The dried extracts

were stored at 8°C in a refrigerator until required.

Table 1: Percentage yield of crude extract of Aloe nuttii baker and aloe chabaudii in various solvents

Aloe Species	Extraction Solvent	Weight of Gel	Weight of Extracts
Aloe nuttii Baker	Distilled Water	100 g	60g
	Ethanol	100 g	62g
	Methanol	100 g	63g
Aloe Chabaudi	Distilled Water	100 g	60g
	Ethanol	100 g	61g
	Methanol	100 g	62g

Evaluation of Antimicrobial Activity
Test Microorganisms

All microbial strains were obtained from the Department of Microbiology at the University Teaching Hospital, Lusaka, Zambia. Stock cultures of Gram-positive bacteria (*Staphylococcus aureus* and *Staphylococcus spp*), and Gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) were subcultered and maintained in nutrient broth at 4°C. The yeast fungi *Candida albicans* was maintained in Sabourauds media at 4°C.

Evaluation of Antimicrobial Activity

The antimicrobial activity of *Aloe nuttii* Baker and *Aloe chabaudii* Schonland extracts were determined by disc diffusion method using a modified Kirby-Bauer disc diffusion method (Bauer et al., 1966). Bacterial strains were cultured in nutrient broth (37°C) for 24 hours. The bacterial strains in the broth each reproduced to contain approximately 10^8 - 10^9 cells mL^{-1}. About 100 µL of each microbial suspension was then spread on Mueller Hinton agar (HiMedia). Sabouraud dextrose broth was used to subculture the *Candida albicans* at 30°C for 48 hours. The fungal culture reproduced to contain approximately 10^5 - 10^6 cells mL^{-1}. Approximately 100 µL of the *C. albicans* culture was then spread onto Sabouraud's dextrose agar. Whatman No. 1 filter paper was used to produce sterile 6 mm diameter paper discs, which were partitioned into three separate sets, each of which was impregnated with 10µL of either methanol extract, ethanol extract or distilled water extract for each of the two *Aloe* species.

RESULTS

Mean Growth Inhibition Zone for Aloe nuttii Baker

In the case of *Aloe nuttii* Baker, the aqueous extracts showed the highest activity against all organisms tested (**Figure 1).** The largest zone of inhibition was observed against *C. albicans* species (13 mm), followed by *S. aureus* (12 mm), *E. coli* (11 mm), *S. aureus* isolates (10.4 mm) and *P. aeruginosa* (9.5 mm). The ethanol, methanol and distilled water extracts showed no activity.

***Aloe Chabaudii Schonland* Growth Inhibition Diameter Zones**

Aloe chabaudii Schonland water, ethanolic and methanolic extracts (**Figure 2)** showed no activity against all the test bacteria (*C. albicans*, *S. aureus*, *E. coli*, *S. aureus* isolate, and *P. aeruginosa*).

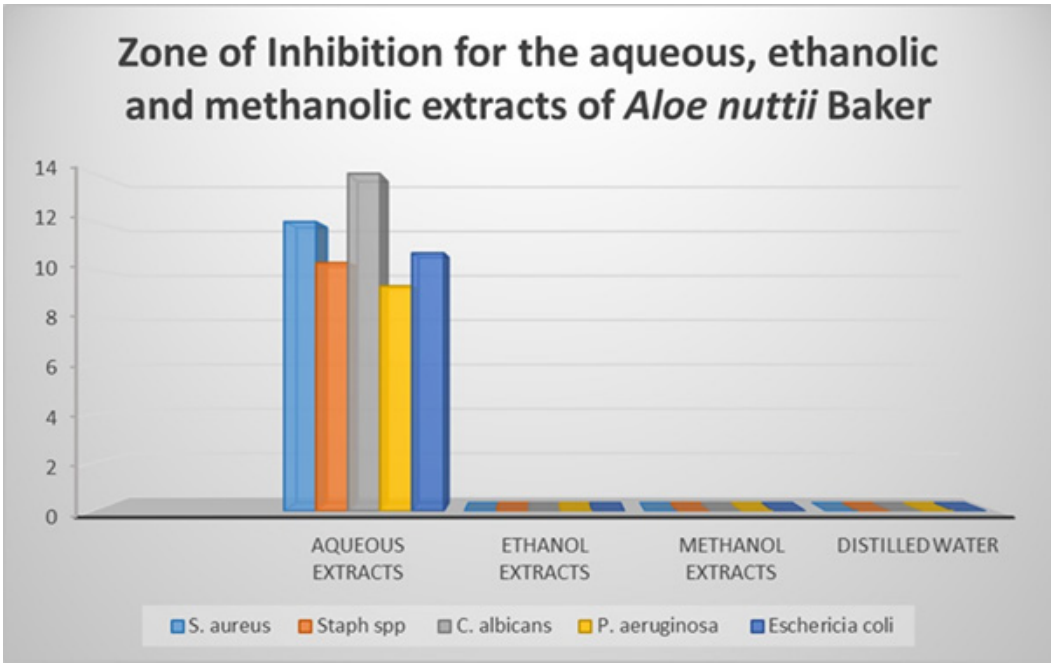

Figure 1: Mean Growth Inhibition Zone for Aloe Nuttii Baker

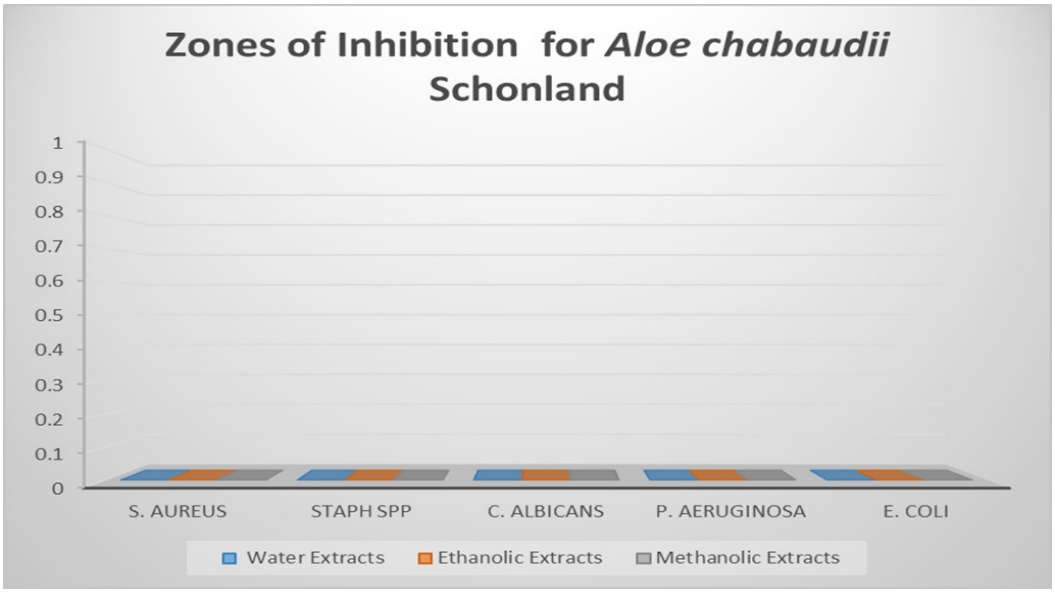

Figure 2: Aloe Chabaudii Schonland Growth Inhibition Diameter Zones

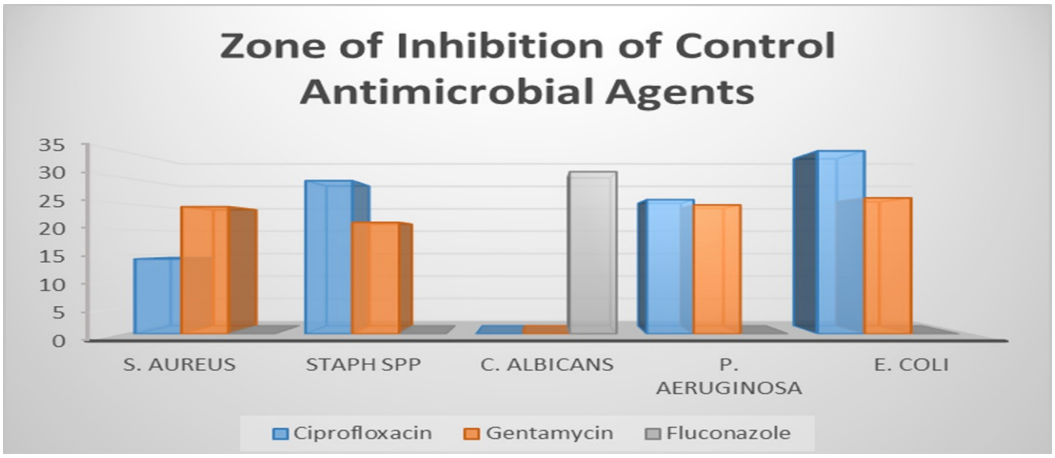

Figure 3: Mean Growth Inhibition Zone Diameters of Positive Controls – Ciprofloxacin, Gentamycin and Fluconazole

Mean Growth Inhibition Zone Diameters of Positive Controls – Ciprofloxacin, Gentamycin and Fluconazole

The positive controls showed remarkable activity against the test organisms, as shown in **Figure 3**. Ciprofloxacin showed zones of inhibition as follows: *S. aureus* (15 mm), *S. aureus isolates* (30 mm), *C. albicans* (no activity), *P. aeruginosa* (26 mm), *E. coli* (35 mm). Fluconazole showed no activity against all test organisms except for *C. albicans* (33 mm). Gentamycin showed good activity against all organisms except *C. albicans,* with the highest activity observed for *E. coli* (27 mm), followed by *P. aeruginosa* (25 mm), *S. aureus* (25 mm) and *S. aureus* isolate (22 mm).

Zones of Inhibition for *Aloe nuttii Baker* Water Extracts vs Negative Controls (Methanol, Ethanol, Water)

As already demonstrated (**Figure 1**), the aqueous extracts of *Aloe nutti* Baker showed activity against all the test organisms, while the methanol and ethanol extracts showed no activity. Paradoxically, the neat ethanol and methanol negative controls both showed activity (**Figure 4),** unlike the distilled water, which showed no activity.

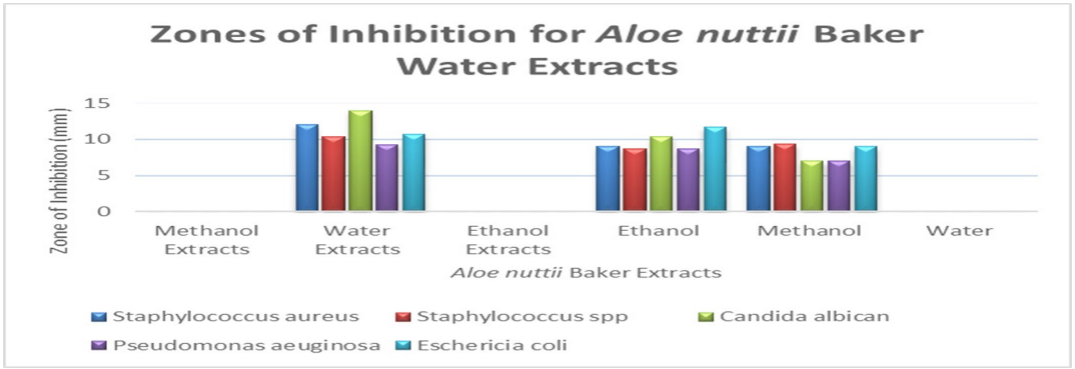

Figure 4: Zones of Inhibition for Aloe Nuttii Baker Water Extracts vs Negative Controls (Methanol, Ethanol, Water)

Minimum Inhibitory Concentration (MIC) for *Aloe Nuttii*

The MIC was 3 mg/ml in the *S. aureus* and was undetermined for the *S. aureus* isolate. The aqueous extracts showed MICs of 1.5 mg/ml each for *C. albicans, P. aeruginosa* and *E. coli,* respectively, as shown in **Figure 5**.

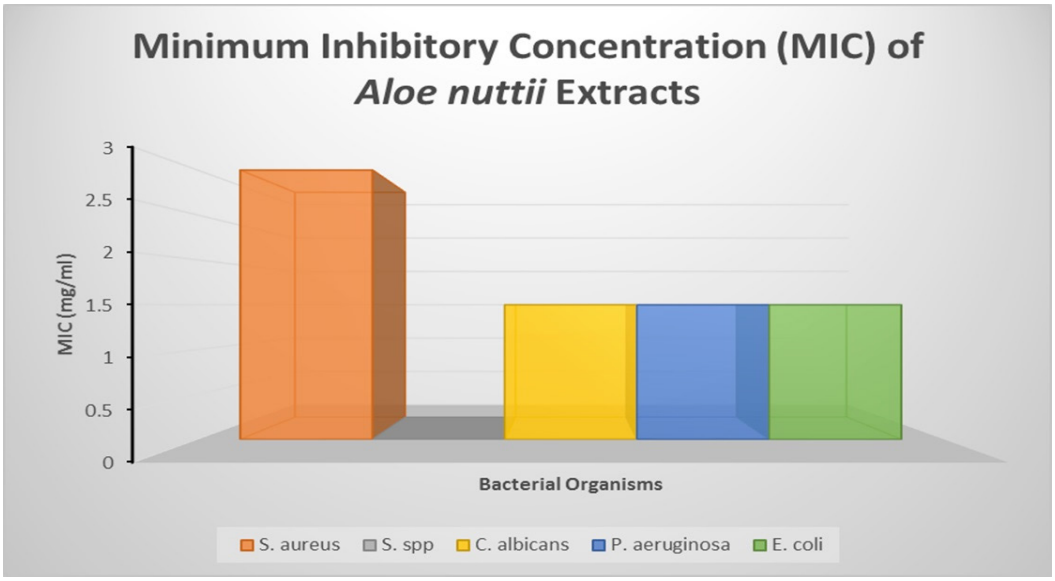

Figure 5: Minimum Inhibitory Concentration (MIC) for *Aloe Nuttii*

DISCUSSION

The antimicrobial activity of Aloe *nuttii* Baker and *Aloe chabaudii* Schonland was investigated by agar disc diffusion method against bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*) and fungi (*Candida albicans*). The leaf extracts in distilled water, methanol and ethanol were tested for their antimicrobial properties against the bacteria and fungi. While distilled water was used as the negative control, commercial antimicrobial agents (Fluconazole 31 mm (22mg/ml; Ciprofloxacin 27 mm (2mg/ml) and Gentamycin 28.5 mm (40mg/ml)) were used as positive controls and were observed to possess more potent antimicrobial activities than the leaf extracts from both *Aloe* species with zones of inhibition shown in **Figure 3**. The water leaf extracts of *Aloe nuttii* Baker showed antimicrobial activity against the gram-positive organism, the two gram-negative organisms tested and the *C. albicans*. *Aloe chabaudii* Schonland did not demonstrate any antimicrobial activity.

No antimicrobial activity was detected for both the methanolic and ethanolic extracts of *Aloe nuttii* Baker and *Aloe chabaudii* Schonland against all tested organisms. The highest zone of inhibition was observed for the water extracts against *C. albicans* (14 mm), and the lowest activity was seen for *P. aeruginosa* (9.3 mm). *S. aureus* and *E. coli* had zones of inhibition of 12 mm and 10.7 mm, respectively, while

that of the *Staphylococcus* isolate was 10.3 mm. Compared to the standard antimicrobial drugs, the distilled water plant extracts showed reasonable inhibition zones, which supports the use of and provides a scientific basis for the traditional therapy of microbial diseases.

Despite reports in the literature suggesting that ethanolic extracts of *Aloe vera* possess more antimicrobial activities compared to water or hexane, this study showed that only the water extracts of *Aloe nutti* Baker had antimicrobial activity [26].

CONCLUSION

Aloe nutti Baker demonstrated promising activity, requiring further study to isolate the active compounds, but in contrast, *Aloe chabaudii* Schonland did not show activity. The usefulness of the *Aloe* leaf gel in the treatment of infections in folk medicine has been demonstrated. However, more work is required to explore the medicinal potential of the plant. To our knowledge, this is the first report of the antimicrobial activity of water extracts of *Aloe nutti* Baker, bringing to the fore the need for follow-up work to conduct phytochemical screening and compound identification to distinguish the actual active compounds that may be presented for optimisation in drug discovery programmes.

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