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*Full Length Research Paper*

# Screening of *Siphonochilus aetiopicus* (Schweinf.) B. L. Burtt for antibacterial and antifungal properties

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***Siphonochilus aethiopicus*** **is often used by traditional healers as a remedy to cure colds, coughs, influenza, etc. A validation of its microbial properties from extracts obtained from leaves and rhizomes were attempted against five gram positive and four gram negative bacteria. Antibacterial and antifungal activities were noted in the ethanol extract of the rhizomes of *S. aethiopicus*. Extracts from the leaves also exhibited antibacterial and antifungal activities in all treatments against the selected bacterial and fungal strains. In this paper, a comparative analysis of the antibacterial and antifungal properties of both rhizomes and leaves was attempted.**

**Key words:** *Siphonochilus aethiopicus*, wild ginger, antimicrobial, traditional medicines.

## INTRODUCTION

Traditional healing plays an integral part of black African culture with the majority of people consulting traditional healers (Lindsey et al., 1999). The demand for traditional plants is therefore high leading to their exploitation from the wild. This exploitation may lead to extinction of many fundamentally important species.

*Siphonochilus aetiopicus*, also know as wild ginger, is an indigenous plant to Southern Africa, widely known for its medicinal properties and currently is on the endangered list. Wild ginger has been harvested to the point of extinction in KwaZulu-Natal and demand for this plant continues because of its high medicinal value. Scientific evidence has brought about the possibility of the utilization of plant extracts in the treatment of fungal and bacterial infections, and the development of antibacterial and antifungal products (Farnsworth, 1994; Fox, 1999). Furthermore, antibacterial activity has allowed a better understanding of the use of traditional medicines as opposed to modern day drug therapies (Coopoosamy and Magwa, 2007). The rhizomes of *S. aethiopicus* is often chewed as well as brewed in the form of a tea for the treatment of a number of ailments such as colds, coughs, influenza etc, by traditional healers (Hutchings et al., 1996). Antibacterial and cyclooxygenase activities have been demonstrated by researchers using ethanol extracts from the rhizome of wild ginger (Lindsey et al., 1999; Stafford et al., 2005). Although, most pharmacological studies have focused on extracts from the rhizomes (Lindsey et al., 1999; Stafford et al., 2005) of wild ginger, little attention as been given to the antifungal and antibacterial properties of the leaves.

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## MATERIALS AND METHODS

### Antibacterial assay

Traditional healers tend to use water (being most polar) for extractions. Less polar media such as ethyl acetate and acetone selectively isolate the less polar compounds and provide a form of separation. Leaf and rhizome material of *S. aethiopicus* was collected from the field and dried in an oven at 60°C until sufficiently dried. One kilogram of dried material of each (stem and rhizome) was then crushed and placed in a 2 L conical flask containing one of three mediums, that is, water, ethyl acetate and acetone, for extraction. The media were left for 72 h in an orbital shaker at 20 shakes per minute. After 72 h the extracts were filtered. The extracts were then used for further tests. The plant extract was then tested for antibacterial properties against five strains of gram-positive (*Bacillus subtilis, Micrococcus kristinae, Bacillus cereus, Staphylococcus aereus* and *Staphylococcus epidermidis*) and four strains of gram-negative bacteria (*Escherichia coli, Proteus vulgaris, Enterobacter aerogenes* and *Shigella sonnei*) for antibacterial activity. Each organism was prepared by diluting in

**Table 1.** Minimal inhibitory concentration (MIC) of *S. a*ethiopicus antibacterial assay on crude extract (Controls: Chloramphenicol and Streptomycin sulfate) n = 3.

| Bacteria | Gram | Medium (MIC) (mg/ml) | | | | | | Control (µg/ml) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Water | | Ethyl acetate | | Acetone | | | |
| $10^6$ Bacteria/ml | +/- | Rhizome | Leaves | Rhizome | Leaves | Rhizome | Leaves | Chlor[a] | Strept[b] |
| *B. subtilis* | + | Na | Na | 4.0 | 5.0 | 3.0 | 4.0 | < 2.0 | < 2.0 |
| *M. kristinae* | + | Na | Na | 4.0 | 6.0 | 3.0 | 5.0 | < 2.0 | < 2.0 |
| *B. cereus* | + | Na | Na | 3.0 | 5.0 | 4.0 | 5.0 | < 2.0 | < 2.0 |
| *S. aereus* | + | Na | Na | 4.0 | Na | 3.0 | Na | < 2.0 | < 2.0 |
| *S. epidermis* | + | Na | Na | Na | Na | 2.0 | Na | < 2.0 | < 2.0 |
| *E. Coli* | - | Na | Na | Na | Na | 3.0 | Na | < 2.0 | < 2.0 |
| *P. vulgaris* | - | Na | Na | Na | Na | 4.0 | Na | < 2.0 | < 2.0 |
| *S. sonnei* | - | Na | Na | Na | Na | Na | Na | < 2.0 | < 5.0 |
| *E. aerogene* | - | Na | Na | Na | Na | Na | Na | < 2.0 | < 2.0 |

Na = No Activity, All tests were done in triplicates and the averages are indicated, Chlor[a] = Chloramphenicol, Strept[b]= Streptomycin sulphate.

24 h old broth cultures with sterile nutrient broth. The cultures were then diluted 100 fold to give approximately $10^6$ bacteria $ml^{-1}$.

#### Antifungal assay

*S. aethiopicus* leaf and bulb (approximately 1kg of each) were cut into small pieces and crushed in a homogenizer. The plant materials were soaked in ethanol (95% v/v) and in distilled water in 2 L conical flasks for 3 weeks. The extracts (water and ethanol) obtained were evaporated at reduced pressure (45°C) to a residue. Extracts for testing ethanol and aqueous extract were prepared in three different concentrations. The stock solutions were prepared by dissolving 100 mg of dry extract in 1 ml of ethanol and water separately in order to obtain a concentration of 100 mg/ml dilutions (1:10, 1:100 and 1:500). These stock solutions were then used in phosphate buffer at pH 6.0 to evaluate the antifungal activity (Champion et al., 1992). The solutions were then tested for antifungal activity using the following fungal cultures: *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes* and *Trichophyton rubrum.* Plates containing potato dextrose agar were used to serve as controls.

## RESULTS AND DISCUSSION

Gram positive bacteria often cause human diseases such as colds, wounds and sores (Waihenya et al., 2002). All gram positive bacteria were inhibited by the ethyl acetate extracts obtained from the rhizome, except for *S. epidermis* (Table 1). However, the acetone extracts derived from the rhizome inhibited all gram positive bacteria. Both ethyl acetate and acetone extracts obtained from leaf samples inhibited all gram positive bacteria except *S. aereus* and *S*. *epidermis* (Table 1). *E. coli* and *P. vulgaris* were the only gram negative bacteria which were inhibited by acetone extracts obtained from the rhizome. This may suggest that, in general, acetone may be a better extraction agent than ethyl acetate.

In contrast, there was no inhibitory effect both in gram-positive and gram negative bacteria in aqueous extracts. It is assumed that the traditional healers were able to obtain the active compounds by boiling large quantities of material in order to unlock the active compounds. The results of the present study on the effect of antibacterial and antifungal activity are in line with those from previous screenings of medicinal plants for antibacterial activity, where most of the extracts of various plant species showed activity against gram-positive strains only (Coopoosamy and Magwa, 2007; Grierson and Afolayan, 1999; Kelmanson et al., 2000; Rabe and Staden, 1997; Shamim et al., 2004; Vlietinck et al., 1995).

The minimum inhibitory concentration values obtained in the current study are relatively high. High minimum inhibitory concentrations (MIC) were obtained for the gram negative *E. coli* and *P. vulgaris* for the acetone extract derived from the rhizome when compared to the control. Ethyl acetate and acetone extracts derived from the leaf also exhibited high MIC values for the gram positive bacteria; *B. subtilis*, *M. kristinae* and *B. cereus* (Table 1). This could be due to the active compounds in the extracts being present in relatively low concentrations. The leaf extracts of *S. aethiopicus* showed similar activities to that of the rhizome. This would indicate a possibility of some transport mechanism of the active ingredients occurring to enable such antibacterial activities to occur both in the rhizomes and leaves. However, the leaf antibacterial activity is lesser than that of the rhizome. This can be explained by the fact that the leaf is primarily involved as a production centre, which in turn, through transport mechanisms, sends the formulated products to the rhizomes, where it is stored. The antifungal activity (Tables 2 and 3) of the ethanol extracts was found to be more effective than aqueous extracts in both the bulb and leaf extracts. However, less activity was shown against *C. albicans*. Growth inhibition (zone of inhibition) was recorded as very high (++++), high (+++), medium (++) and low (+), which indicated zones of inhibition between 41 - 50, 31 - 40, 21 - 30 and 11 - 20 mm, respectively. The ethanol extract

**Table 2.** Effect of ethanol and aqueous extracts obtained from *S. aethiopicus* on different fungal species.

| Fungal species | Ethanol extract | | | | | | Aqueous extract | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | L | R | L | R | L | R | L | R | L | R | L |
| | 1:10 | 1:10 | 1:100 | 1:100 | 1:500 | 1:500 | 1:10 | 1:10 | 1:100 | 1:100 | 1:500 | 1:500 |
| *A. flavus* | ++++ | +++ | +++ | + | ++ | + | ++++ | ++ | +++ | + | ++ | - |
| *A. glaucus* | ++++ | ++ | +++ | + | ++ | + | +++ | + | ++ | + | + | - |
| *C. albicans* | +++ | ++ | +++ | + | ++ | + | +++ | + | +++ | _ | ++ | - |
| *C. tropicalis* | +++ | ++ | ++ | + | ++ | + | +++ | + | ++ | + | ++ | + |
| *T. mentagrophytes* | +++ | + | ++ | + | ++ | + | ++ | + | ++ | + | ++ | + |
| *T. rubrum* | +++ | + | ++ | + | ++ | + | ++ | - | + | - | - | - |

- = Negative antifungal activity, R = Rhizome, L = Leaves
+ = Positive antifungal activity (low inhibition)
++ = Positive antifungal activity (medium inhibition)
+++ = Positive antifungal activity (high inhibition)
++++ = Positive antifungal activity (very high inhibition)
N.B. Plates containing Potato dextrose agar only served as controls. Control did not show any inhibition of any of the test fungal species.

**Table 3.** Minimal inhibitory concentration observed in different concentrations prepared from stock solution of 100 mg/ml of aqueous and ethanol extracts of *S. aethiopicus* n = 3.

| Fungal species | Ethanol extract | | Aqueous extract | |
|---|---|---|---|---|
| | Bulbs | Leaves | Bulbs | Leaves |
| *A. flavus* | 1:500 | 1:500 | 1:500 | 1:500 |
| *A. glaucus* | 1:500 | 1:500 | 1:500 | 1:500 |
| *C. albicans* | 1:100 | 1:500 | 1:500 | 1:500 |
| *C. tropicalis* | 1:500 | 1:500 | 1:500 | 1:500 |
| *T. mentagrophytes* | 1:500 | - | - | - |
| *T. rubrum* | 1:500 | - | - | - |

Number of replications = 3.

of both the leaves and rhizomes were noted to possess more antimicrobial effects as compared to the aqueous extracts. The high zones of inhibition noted in the ethanol extracts (using a 1:10 concentration) suggest further explanation of the possibility of using this plant against certain ailments caused by the above fungal organisms.

## Conclusion

It is evident that both the rhizomes and leaves exhibit antibacterial and antifungal activities. However, the antimicrobial activities of the leaf are much lower than that of the rhizome extracts. Aqueous extracts of the leaves and rhizomes may not be effective against bacterial growth however; it does inhibit the growth of the fungal species in Table 2. Although the antimicrobial activity in leaves is more limiting, it could assist in reducing the use of the rhizomes only in traditional treatments thus ensuring a more sustainable use of *S. aethiopicus*.

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