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In vitro Antibacterial activity and Phytochemical screening of the Zimbabwean endemic *Aloe orthlopha* Christian & Milne-Readhead (Aloaceae).

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ABSTRACT

Methanol leaf extracts of a Zimbabwean endemic plant *Aloe orthlopha* were investigated for their phytochemical profile and antibacterial properties. Qualitative phytochemical analyses revealed the presence of tannins, terpenoids, reducing sugars, saponins and flavonoids and absence of alkaloids, anthraquinones and cardiac gylcosides. Antibacterial activity of the extract was evaluated on four clinical bacteria using the agar disk diffusion method. Extracts showed activity against *Pseudomonas aeruginosa*, *Salmonella enterica* and *Staphylococcus aureus* and no activity against *Corynebacterium diphtheriae*. *P. aeruginosa* showed the highest susceptibility to the extract with a diameter zone of inhibition of ±13.5mm, then *S. enterica* (±11mm) and *S. aureus* (±9.5mm). *C. diphtheriae* was resistant to the extract. Minimum inhibitory concentration (MIC) values were high ranging from 133 mg/ml in *P. aeruginosa, S. enterica and S. aureus* to 267 mg/ml in *C. diphtheriae*. The results of this study reveal the presence of several phytochemicals with proven antibacterial properties in *A. orthlopha*, thus validating the traditional uses of the plant in treating bacterial infections.

Keywords: *Aloe orthlopha, endemic, phytochemicals, antibacterial activity, clinical bacteria*.

1. INTRODUCTION

The genus *Aloe* L. comprises approximately 420 species with centres of diversity in southern and east Africa, the Arabian Peninsula and Madagascar [1]. The genus is an important source of biologically active compounds with well over 130 phytoconstituents isolated from the group [2]. The harvesting of Aloes for their natural products has presented a threat to their survival in the wild and this has resulted in all *Aloe* species with the exception of *Aloe vera*, being protected by various national and international conventions. Major phytochemical and pharmacological studies on Aloes have focussed on the 'drug aloes' i.e. *Aloe vera* and *Aloe ferox*, two species of immense commercial interest in the pharmaceutical and cosmetics industries [3]. In Zimbabwe, 29 species have been recorded [4], some of which are used in traditional medicine [5, 6].

Aloe orthlopha is endemic to Zimbabwe where it is confined to serpentine soils of the northern part of the Great Dyke mountains. The species is a perennial stemless herb with a dense rosette of succulent leaves. The leaf lamina is greenish grey, erect to sub-erect, wide towards the base, and pinkish tinged. The leaf margin is pinkish, with pungent triangular shaped light brown teeth. The inflorescence is erect, largely solitary, with horizontally spreading branches bearing densely flowering racemes. Flowers are reddish to orange, and are held on the upper side of the raceme.

The principal therapeutic uses of *Aloe* species in Zimbabwe traditional medicine are to treat sexually transmitted infections, wounds, diarrhoea, constipation, asthma, abdominal pains, and poultry diseases [5, 7]. *A. orthlopha*, although reportedly poisonous [5] is known to be used as a medicinal plant by traditional healers [6]. The species has not been pharmacologically evaluated. The importance of the country's diverse *Aloe* flora lies not only in their therapeutic value in traditional healthcare but also in their potential as sources of new chemical constituents for drug discovery.

This study evaluates the phytochemical profile and antibacterial activity of *A. orthlopha* to assess its use in traditional medicine.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

A. orthlopha leaves were freshly collected from Mutorashanga Pass along the Great Dyke of Zimbabwe in January 2012. The plant was identified in the field, and a voucher specimen was deposited at the University of Zimbabwe teaching herbarium.

2.2 Preparation of Plant Extract

Fresh *A. orthlopha* leaves were peeled to remove the outer rind. The mucilaginous inner pulp was minced and thoroughly homogenised in a blender. Some 60 ml of homogenate were mixed with 200 ml analytical grade methanol in a thimble and extracted using a Soxhlet apparatus for 24 hours. The crude extract was then concentrated using a rotary evaporator at 40^0C under reduced pressure.

2.3 Screening of Phytochemical Components

Standard qualitative methods [8, 9] were adopted for phytochemical screening. The crude extract was tested for phytochemical constituents using the following tests and reagents: reducing sugars with Fehlings test, anthraquinones with Borntragor's test, terpenoids with

Salkowski test, flavonoids with ammonia and suphuric acid, saponins with foam test, tannins with Ferric Chloride test, alkaloids with Mayer's and Draggendorff's tests and cardiac glycosides with Keller- Killian's test.

2.4 Evaluation of Antibacterial Activity

2.4.1 Bacterial strains

The test organisms included both gram positive (*Corynebacterium diphtheriae*, *Staphyloccoccus aureus*) and gram negative bacteria (*Pseudomonas aeruginosa*, *Salmonella enterica)*. These were obtained from clinical cultures obtained from the University of Zimbabwe's Medical School.

2.4.2 Agar Paper Disk Diffusion Method

Antibacterial activity of the *A. orthalopha* extract was determined by the Agar Disk Diffusion Method [10]. Nutrient agar and nutrient broth (DIFCO) were prepared according to the manufacturer's instructions. The bacterial isolates were cultured in nutrient broth for 18 h at 37 ^{o}C in a shaker incubator. Following incubation, the cultures were washed twice using sterile 0.9% saline solution. The cultures were centrifuged at 3000 rpm for 10 minutes, using a Heraeus labofuge 200 centrifuge. The supernatant was decanted, and the pellet re-suspended in sterile 0.9% saline solution. Bacterial suspension was re-centrifuged, decanting the supernatant again to obtain a bacterial pellet. The pooled bacterial pellet was finally resuspended in 0.9% saline solution and brought to a turbidity of 0.5 McFarland Standard. At this turbidity the bacterial suspension contains approximately 1.5 x 10^{8} cfu/ml. The McFarland standard was prepared by mixing 0.05 ml of 1.175% barium chloride dihydrate with 9.95 ml of 1% sulphuric acid [11].

200 µl of each bacterial suspension was uniformly spread onto the sterile nutrient agar plates. Whatman filter paper (No: 1) was used to prepare disks approximately 6 mm in diameter. The paper disks were impregnated in 400 mg/ml of the plant extract. Blank disks were impregnated with the following positive controls: Tetracyclin (30 mg/ml), Chloramphenicol (30 mg/ml), Streptomycin (10 mg/ml) and Penicillin G (10 mg/ml). Paper disks impregnated with distilled water served as negative controls. The disks were asceptically transferred to nutrient agar plates pre-inoculated with the respective bacteria. All the experiments were replicated twice and the plates were incubated for 24 h at 37° C. The antibacterial activity was assessed by measurement of inhibition zones.

2.4.3 Determination of Minimum Inhibitory Concentration (MIC)

Determination of the minimum inhibitory concentration (MIC) was carried out following the method of [12]. Plant extract concentrations of 400mg/ml, 200mg/ml, 100 mg/ml and 50 mg/ml were prepared by dilution in distilled water. In triplicates, 0.5 ml of each extract concentration were pipetted into test tubes containing 0.5 ml of nutrient broth. The test organism (0.5 ml) was pipetted into each of the test tubes containing the mixture of the broth and extract, and incubated at 37^{0}C for 18 hours after which it was observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

3. RESULTS

3.1 Phytochemical Screening

The result of the phytochemical screening revealed the presence of tannins, terpenoids, reducing sugars, saponins and flavonoids (Table 1). Tests for reducing sugars, alkaloids and anthraquinones yielded negative results.

Table 1: Results of Phytochemical Screening Tests on Extract of A. *Orthalopha.*

Phytochemical test	Observation
Terpenoids	+
Alkaloids	-
Saponins	+
Flavonoids	+
Anthraquinones	-
Cardiac glycosides	-
Tannins	+
Reducing sugars	+

Key: += present- = absent

3.2 Antibacterial activity

Table 2 shows the diameter zones of inhibition of *A. orthalopha* leaf extracts against the test bacteria. Extracts showed activity against *P. aeruginosa*, *S. enterica* and *S. aureus* and no activity against *C. diphtheroides*. The Gram-negative bacteria, *P. aeruginosa* and *S. enterica* were more susceptible to extract inhibition when compared to the Gram-positive bacteria tested. The antibacterial activities of the methanol extract were generally less than the standard antibiotics.

Table 2: Growth Inhibition Zones (mm) of *A. Orthrolopha* Leaf Extracts on Growth of Different Bacteria species
(means ± SD, n = 2).

	P. aeruginosa	*S. aureus*	*S. enterica*	*C. diphtheroides*
Methanol Extract	13.5±0.71	9.5±0.71	11.0	0
Chloramphenicol	22.5±0.71	24±4.24	35.5±0.71	36.5±0.71
Penicillin	11±1.41	19±1.41	26.5±0.71	30
Streptomycin	31.5±2.12	27	28.5±0.71	30
Tetracycline	24.5±0.71	15.5±4.95	21.5±4.95	28.5±2.12
Distilled water	0	0	0	0

Table 3 shows the minimum inhibitory concentrations (MICs) for the extract against the test organisms. MIC values shown were calculated from the concentration of extract in the final volume in the nutrient broth test tubes.
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Table 3: Minimum Inhibitory Concentration (MIC) of *A. Orthrolopha* Leaf Extract.

Species	MIC (mg/ml)
P. aeruginosa	133
S. enterica	133
S. aureus	133
C. diphtheroides	267

4. DISCUSSION

Phytochemical screening of the crude extracts of *A. orthrolopha* revealed the presence of bioactive phytochemicals (Table 1). Studies on other *Aloe* species like *A. vera* [13, 14), *A. ferox* [15], *A. purpurea*, *A. tormentorii*, *A. macra* [16] yielded similar phytochemical profiles. Several authors have linked the presence of these compounds to pharmacological properties of crude plant extracts [17, 18]. Tannins and flavonoids are phenolic compounds known to exhibit activities that include antimicrobial, anti-inflammatory, analgesic and anti-oxidant properties [19]. Flavonoids function by altering the activity of enzymes involved in cell division, platelet aggregation and immune system responses and complex with bacterial cell wall. Tannins precipitate microbial protein rendering it unavailable to bacteria [20]. Tannin containing plants are astringent in nature and are used to arrest bleeding and for treating wounds and intestinal disorders such as diarrhoea and dysentery, thus exhibiting antibacterial activity [21]. Saponin antibacterial activity was established by a number of researchers [22, 23, 24]. Some reports indicate that antibacterial activity is due to total saponin and tannin content [25]. Terpenoids are also reported to have antibacterial properties and their activity is through disruption of bacterial membranes [26].

This study showed that *A. orthrolopha* leaf extracts have antibacterial activity and that the susceptibility of bacteria to plant extracts varied according to species. The degree of antibacterial activity of the leaf extract was assessed based on the size of the diameter zones of inhibition according to recommendations by [27], i.e. halos <9 mm, inactive; 9 to 12 mm, with little activity, 13 to 18 mm, active,> 18 mm very active. Following these recommendations the extracts were active against *P. aeruginosa* (halos>13 mm), showed little activity against *S. aureus* and *S. enterica* (halos 9 -12 mm) and were inactive against *C. diphtheroides* (halos<9 mm). The fact that the extracts were active against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity of the extract. This observation is very significant because of the possibility of developing therapeutic drugs that will be active against multidrug-resistant bacteria. The observation that the extract shows activity against the Gram-negative *P. aeruginosa* is also significant since Gram-negative bacteria are known to be resistant to the action of most anti-bacterial agents including plant based extracts [28]. Gram-negative bacteria possess an outer phospholipid membrane with lipopolysaccharide components which make their cell wall impermeable to antimicrobial agents [29]. When compared to the antibacterial activity of *A. vera* against *P. aeruginosa* and *S. aureus* [13, 14, 16], the results obtained in this study show smaller zones of inhibition for the *A. orthrolopha* leaf extract. This could mean the *A. orthrolopha* leaf extract is less active than the *A. vera* extracts against the tested bacteria.

Plant extracts are generally considered of pharmacological interest if minimum inhibitory concentration values are less than 1 mg/ml [30]. All the MIC values for *A. orthrolopha* leaf extracts obtained in this study were above 1 mg/ml and

may therefore be considered of little pharmacological importance against the test bacteria. This, however, does not disqualify *A. ortholopha* as a potential source of effective antibacterial agents as the extracts could have contained low concentrations of the concerned phytochemicals. Additionally phytomedicines are known to act synergistically [31], so it may be possible that some chemical constituents may not have been extracted by the solvent used. [32] discourages use of high temperatures during Soxhlet extraction as this may lead to degradation of thermolabile compounds in the extract.

5. CONCLUSIONS

The results of the study showed the presence of antibacterial phytoconstituents in *A. ortholopha* thus supporting the traditional use of the plants. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

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