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Antimicrobial activity of aqueous and methanol extract of naturally growing and cultivated *Aloe turkanensis*

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Abstract

The aim of the study was to determine the antibacterial properties of aqueous and methanol extracts of naturally occurring and cultivated *Aloe turkanensis*. The plant is widely used as a traditional herb by communities in Turkana County, Kenya. However its efficacy has not been established. Aqueous and methanol extract of a naturally occurring whole *Aloe turkanensis* and cultivated one was obtained. The extracts were tested for in-vitro activity against 4 standard bacterial cultures and a fungal clinical isolate. Benzyl penicillin, Gentamycin and Amphotericin B were used as positive controls. Efficacy data analysis showed that methanol extracts of naturally growing plant inhibited the growth of *B. cereus* (100 mg/ml), *S. aureus* (100 mg/ml), and *P. aeroginosa* (200 mg/ml) with mean diameters of inhibition zones for *S. aureus* and *B. cereus* being 18.5±0.7 mm and 16.5±0.7 mm, respectively. Aqueous extract of the plant inhibited the growth of *B. cereus* and *S. aureus* at a Minimum Inhibitory Concentration of 200 mg/ml and 50 mg/ml respectively with mean diameter of inhibition zones for *S. aureus* and *B. cereus* being 19.75±1.0 mm and 11.5±0.0 mm respectively. Methanol extracts of cultivated A. turkanensis inhibited the growth *B. cereus* (100 mg/ml), *S. aureus* (50 mg/ml), *E. coli* (400 mg/ml) and *P. aeroginosa* (200 mg/ml) with mean diameters of inhibition zones for *S. aureus* and *B. cereus* being 18.5±0.7 mm and 11.5±0.0 mm respectively. There was a significant difference in antibacterial activity between the two plant ecotypes ($p<0.05$).

Keywords: *Aloe turkanensis,* Antibacterial, Naturally occurring, Cultivated.

Introduction

Aloe turkanensis is a succulent monocotyledonous plant in the family Asphodelaceae (International Code of Botanical Nomenclature). It is a sprawling shrub with stems of up to 70 cm long. It grows in loose clumps up to 2 m diameter.[1, 2] Figures 1 and 2 are photographs *Aloe turkanensis* taken in its natural and cultivated environs

Turkana community uses the plant for the treatment of eyes diseases, wounds, stomach ache, ringworms, burns and poultry diseases. The juice from boiled roots is added to a drink to induce vomiting, which is said to relieve persistent headaches. However, the antimicrobial properties of this species have not been explored and documented. The aim of this study was to ascertain and validate the use of the plant extract for antibacterial and antifungal activity and investigate whether there is a difference in bioactivity of a naturally occurring plant and a cultivated one. Figure 1(a and b) is photographs of *Aloe turkanensis* taken in its natural and cultivated environs.

(a) **(b)**

Figure 1: A photograph of *Aloe turkanensis* growing; a) in its natural habitat in Turkana County; b) in Karura Forest, Kiambu County

Turkana community uses the plant for the treatment of eyes diseases, wounds, stomach ache, ringworms, burns and poultry diseases. The juice from boiled roots is added to a drink to induce vomiting, which is said to relieve persistent headaches.[3] However; the antimicrobial properties of this species have not been explored and documented. The aim of this study was to ascertain and validate the use of the plant extract for antibacterial and antifungal activity and investigate whether there is a difference in bioactivity of a naturally occurring plant and a cultivated one.

Materials and Methods

Collection of the Plant, Identification and Extraction

The naturally growing *A. turkanensis* plant sample was obtained from Natira community aloe garden at the outskirts of Kakuma town, Turkana County. The County lies between latitude 3^0 37´ North and longitude 36^0 0´ East. The plant was collected during a dry season, identified at Kenya Forestry Research Institute in Karura where voucher specimen was deposited (At/111K). A seedling of the plant was cultivated for eight months in Karura Forest, Kiambu County.

Whole plant materials from the two plant ecotypes' were thoroughly cleaned, chopped into small pieces and aerated to dryness. In a fume chamber, dry plant materials were placed in Cunningham® grinder, ground into powder and placed in clean airtight polythene paper.[4] Two hundred grams of the plant powder were extracted separately. The powdered pant materials were placed in two conical flasks; 70% v/v methanol was added into one flask while distilled water was added into the other until the powders were submerged. The flasks were corked, agitated for 96 hours at room temperature to allow proper percolation and extraction. On the fifth day, the extracts were filtered using Whatman No. 1 filter papers into other conical flasks. Each of the extract was then evaporated to dryness using a rotary evaporator and freeze-dried. The experiments were conducted at the Department of Public Health Pharmacology and Toxicology. Table 1 show a summary of relative percentage yield of the two plant ecotypes following solvent extraction.

Table 1: Extract yield following extraction of the two ecotypes of *Aloe turkanensis*

Plant specimen	Amount of Powder (gms)	Solvent (1:10)	Yield after extraction (gms)
Naturally occurring AT	200	Methanol	14.96
	200	Aqueous (Water)	13.32
	200	Methanol	12
Cultivated AT	200	Aqueous (Water)	15

Testing for *in* vitro antimicrobial activity of the extracts

The preliminary studies were done using Agar well diffusion method. A loopful of stock cultures of standard organisms that were stored in cooked meat media were sub inoculated on BA (Oxoid®) and incubated for 24hours at 37°C. The sub-cultured bacteria were used as stock cultures and were kept refrigerated at +4°C. Using a sterile loop, a single colony was picked and streaked on pre-prepared Mueller Hinton Agar (Oxoid®) and incubated for 18 hours at 37°C. Standard well were made on the agar plate (1cm in diameter). The 50ul of the plant extracts at different concentrations were added into the wells and incubated for 24 hours at 37^0C. Then the diameters of the zones of inhibition were measured.

Broth dilution technique as described by Suffredini[5] was used to determine the minimum inhibitory concentration. On the 1st day, bacterial inoculums of each reference bacterial were obtained from cooked meat media and subcultured in blood agar (Oxoid®) for 24 hours at 37^0C except for *Candida albicans* which was incubated at room temperature. On the 2nd day, a loopful colony was picked using a sterile loop & put into 3ml sterile physiological buffer saline (PBS). Serial dilution was made to density equivalent Macfarland opacity No. 6 Barium Chloride in 1% sulfuric acid to make a concentration of 10^6 cfu/ml. Stock solution of the plant extracts was made by dissolving 4 grams of the powdered extract in 10 ml of normal saline to make a concentration of 400 mg/ml. From this, two fold serial dilutions were made in sterile Muller Hinton Broth in culture tubes. One milliliter of the test organisms' suspension was dispensed into the culture tubes containing the plant extracts. Standard negative controls wells were prepared by adding 1ml of the test organisms' suspension in sterile Muller Hinton Broth. For positive control, Benzyl penicillin, Gentamycin and Amphotericin B were used. Each tube containing the test organism and plant extract was the incubated for 24 hours at 37^0C except for *Candida albicans* which was incubated for 48 hours at room temperature. On the 3rd day, the inhibition of test organisms was evaluated by culturing 1ml of the suspension into Muller Hinton Agar for 24hour at 37^0C for bacteria and for 48 hours at room temperature for *Candida albicans*.

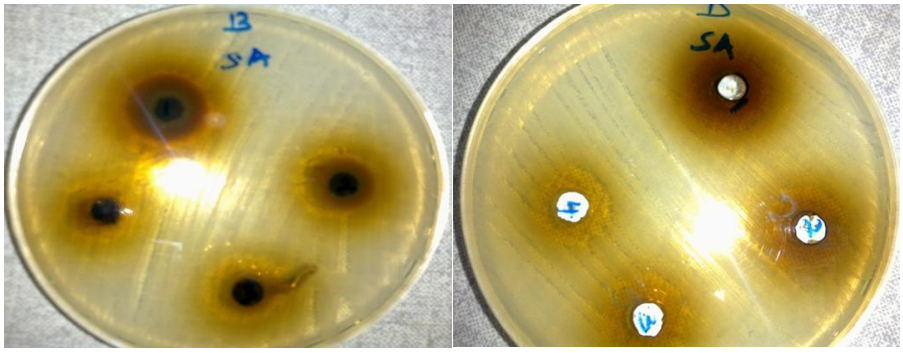
Results

On both broth dilution and agar well diffusion method, the methanol and water extracts were found to have antibacterial activities except for water extract of the cultivated plant. However no antifungal activity against the clinical isolate of *Candida albicans* was noted for both water and methanol extracts even on the highest working concentration of 400 mg/ml. On broth dilution method, aqueous extract of naturally growing *Aloe turkanensis* inhibited *S. aureus* bacteria at a concentration of 50 mg/ml which had a similar activity as methanol extracts from cultivated *Aloe turkanensis*. This activity was not different from the result gotten on a different test (agar well diffusion) where the water extract (400 mg/ml) zone of inhibition for the growth of *S. aureus* was recorded as 19.75±1mm while for methanol it was 18.5±0.7mm. For the methanol extract of naturally growing *Aloe turkanensis*, there was a slight difference in activity compared to water extract after inhibiting the growth of the same bacteria at a concentration 100 mg/ml. However, the activity of methanol extract of cultivated *Aloe turkanensis* against *S. aureus* on agar well diffusion was seen to decrease with zone of inhibition recorded as 16.5±0.7mm.

On broth dilution method, water and methanol extracts activity against *B. cereus* was lower compared to *S. aureus* where the naturally growing *Aloe turkanensis* extracts inhibited the growth of *B. cereus* at the concentration of 200 mg/ml and 100 mg/ml, respectively. The activity of water extract of naturally growing *Aloe turkanensis* was similar to that of methanol extracts of cultivated *Aloe turkanensis*. On agar well diffusion, methanol extracts of naturally growing *Aloe turkanensis* zone of inhibition for *B. cereus* was 16.5±0.7 mm while water extract zone of inhibition was recorded as 11.5 mm.

Methanol extracts of naturally growing *Aloe turkanensis* and cultivated *Aloe turkanensis* inhibited the growth of *P. aeroginosa* at a concentration of 200 mg/ml and 400 mg/ml respectively. However no activity was reported on agar well diffusion. The lowest activity of the plant was reported from methanol extract of cultivated *Aloe turkanensis* plant where it inhibited the growth of *E. coli* at a concentration of 400 mg/ml.

More analysis carried out using Analysis of variance to determine the significance of the differences in biological activity of cultivated and naturally growing plant extracts shows that there was a significant difference ($P<0.05$) in bacterial growth inhibition. Figure 2(a and b) is showing diameter of zones of inhibition of microbial growth for extracts on Agar well diffusion.



(a) **(b)**

Figure 2: A culture plate showing diameter of zones of inhibition of microbial growth for; **a)** aqueous extract; **b)** methanol extract of naturally growing *Aloe turkanensis* against *Staphylococcus aureus*

Table 2 and figure 3 shows a summary of the diameter zones of inhibition noted when methanol and aqueous extract of the two plants ecotypes are tested against 4 standard bacteria at the highest working concentration of 400 mg/ml

Table 2: Diameters of zone of inhibition of microbial growth for aqueous and methanol extract of *Aloe turkanensis* recorded on Agar well diffusion test against various species of micro-organisms

Plant extract/Test Organism	Diameter of zone of inhibition in mm			
	Naturally growing plant		Cultivated plant	
	Methanol extract	Water extract	Methanol extract	Water extract
S. aureus	18.5±0.7	19.75±1.0	16.5±0.7	6
B. cereus	16.5-0.7	11.5	11.5	6
E. coli	6	6	6	6
P. aeroginosa	6	6	6	6

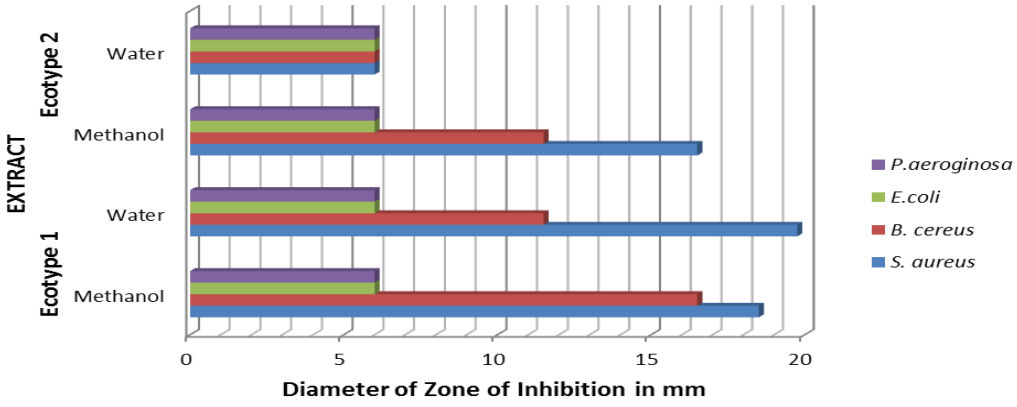


Figure 3: A chart showing diameter of zones of inhibition of microbial growth for aqueous and methanol extracts of Aloe turkanensis against various species of micro-organisms

Table 3 and figure 4 shows a summary of minimum inhibitory concentration when methanol and aqueous extract of the two plants ecotypes are tested against various test organisms.

Table 3: Minimum inhibitory concentrations (MIC) mg/ml for methanol and aqueous extracts different ecotypes of Aloe turkanensis

Test material	Plant material	Extract	*P. aeroginosa*	*E. coli*	*S. aureus*	*B. cereus*	*C. albinans*
A. turkanensis	Naturally growing *Aloe turkanensis*	Aqueous	-	-	50	200	-
		Methanol	200	-	100	100	-
	Cultivated *Aloe turkanensis*	Aqueous	-	-	-	-	-
		Methanol	400	200	50	100	-
Benzyl penicillin		-	-	-	0.625	0.625	
Gentamycin		-	0.0049	0.0049	-	-	-
Amphotericin B		-	-	-	-	-	0.0125

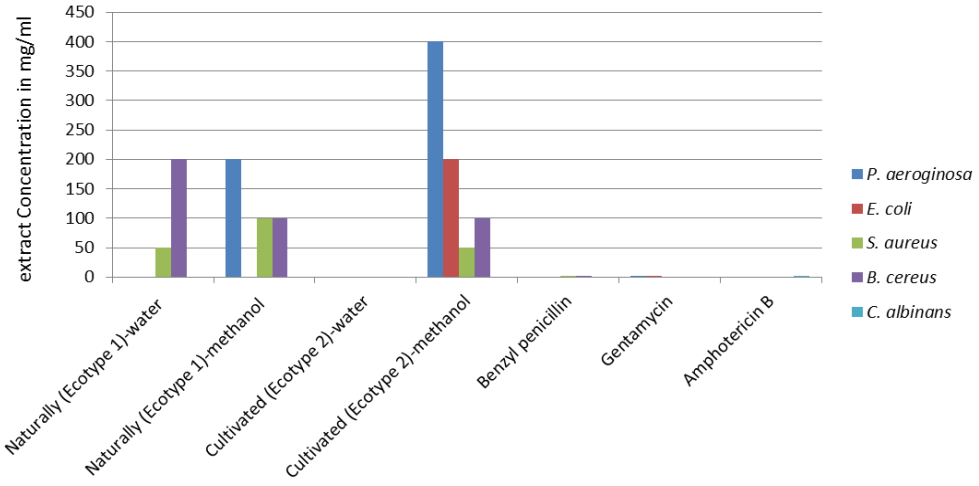


Figure 4: Comparative minimum inhibitory concentrations of cultivated and naturally growing Aloe turkanensis against various species of micro-organisms

Discussion

The results on antibacterial tests indicate that some plants used in traditional medicine have antibacterial activity and lack antifungal activity. The growth of bacterial organism on some crude methanol extracts can be due to natural resistant of the organism or lack of antimicrobial principle. The plant under study was identified by traditional healers as important in the management of infectious diseases. The extracts from naturally occurring *Aloe turkanensis* and methanol extract of the cultivated plant showed a narrow spectrum of antibacterial activity by inhibiting the growth of gram positive bacteria. Their use by TMPs in management/treatment of opportunistic bacteria is therefore justifiable.

However is worth noting that the study shows a variation in extracts yield and biological activity between the two plant ecotype and the solvent used during extraction. The variation in yield and biological activity could be attributed to difference in the type and amount phytochemicals concentrated during growth of the plant. The variation in phytochemicals is an attribute of differences in soil, age, seasons, climate and type of vegetation among the ecological zones.[6] Phytochemical production in plants varies with the geographical location. Plant developmental stage influences secondary metabolism; defense compounds are generally more concentrated and diverse when plants are young and more "apparent" to herbivores, but they are known to decrease with age as structural defenses are developed.[7, 8]

Therefore while exploiting the natural products, scientists need to embrace the concept of sustainability as well as ecological impact on the value of medicinal plants. Many types of action can be taken in favor of conservation and sustainable use of medicinal plants. Some of these are undertaken directly at the places where the plants are found, while others are less direct, such as some of those relating to commercial systems, *ex situ* conservation and bio-prospecting. The results on antimicrobial activity of the two ecotypes under study indicate that there is a difference in the bioactivity expressed when the plants are cultivated in different environment. For this reason, most work by conservationists on medicinal plants should be with those people who own, manage or make use of these species, or else own or manage the land on which they grow. The conservationists need to identify the conditions at field sites that are most favorable for releasing the potential offered by medicinal plants to achieve maximum value of the plant in terms of bioactivity, conservation and sustainable development. It is in working with such stakeholders that the special meanings of medicinal plants to people can best be 'exploited'.[9]

Sustainability requires the consideration of the economic, environmental and social aspects of products and product systems. Therefore, responsible decision-making in public policy, industry and related fields should consider those issues for present and future relevance.[10]

Conclusion

The aqueous and methanol extract of naturally growing and cultivated *Aloe turkanensis* showed appreciable antibacterial properties but lacked antifungal activity against *Candida albicans*. The aqueous extract of naturally growing *Aloe turkanensis* showed a higher antibacterial activity against *S. aureus* compared to methanol extract. Methanol extracts of the cultivated *Aloe turkanensis* showed higher antibacterial activity against *S. aureus* compared to aqueous extract. Aqueous extract of the cultivated *Aloe turkanensis* showed no antibacterial activity.

Acknowledgement

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