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Research Article

Antimicrobial Properties of *Aloe vera, Aloe volkensii* and *Aloe secundiflora* from Egerton University

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Abstract

Disease is the most dangerous man's enemy in this age. This has worsened with the enormous development of drug resistance witnessed in many parts of the world. This study aimed at determining the antimicrobial properties of *Aloe vera, Aloe volkensii* and *Aloe secundiflora* crude extracts. The plants samples were collected from the Department of Biological Sciences, Egeton University. The plants gel was separately extracted by crushing with a pestle and a mortor. The outer green rids and the pulps were separately dried at 50°C in a hot air oven followed by crushing using pestle and mortar. The extraction of the metabolites was carried out using methanol. The phytochemical screening of the crude extracts was carried out to determine the secondary metabolites present in the crude extracts. The antimicrobial tests were carried out using Mueller Hinton agar for bacteria and potato dextrose agar for fungi using agar well diffusion technique. The minimum inhibitory concentration was determined using two fold dilution assay followed by determination of microbicidal and microbistatic properties of the extracts. The samples presented varying levels of saponins, glucosides, flavonoids, tannins, proteins and alkaloids. The extracts form *Aloe vera, Aloe volkensii* and *Aloe secundiflora* inhibited growth of *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Erwinia carotovora, Candida albicans* and *Fusarium oxysporum*. The zones of inhibition varied significantly (F = 3.424051, P = 0.005007) between the extracts of *Aloe vera, Aloe volkensii* and *Aloe secundiflora*. The minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration of the extracts varied significantly (F = 4.696456, P = 0.000534). The three varieties of *Aloe* spp. namely *Aloe vera, Aloe volkensii* and *Aloe secundiflora* were collected from the Department of Biological Sciences, Egerton University. Different crude extracts from them were obtained having varying secondary metabolites. The antimicrobial properties of the crude extracts were tested against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Fusarium oxysporum.*

Keywords: Aloe vera; Aloe secundiflora; Aloe volkensii; Antimicrobial; Egerton; MIC; MBC; MFC

Introduction

The development of dung resistance against convectional medicine has led to people looking for alternative medicine in many countries today [1]. In some developing countries, plants are the main source of treatment against most diseases. Approximately 20% of the world's plants have been pharmacologically tested against majority of pathogenic microorganisms [2]. This has led to introduction of new antibiotics into the drug market [3].

One of the plants that have gained a lot of popularity in medicine is the *Aloe* spp. belonging to the family *Liliaceae* [4]. The plant is succulent with a whorl of elongated, pointed leaves. The term Aloe is derived from an Arabic word 'alloeh' meaning 'bitter' which refers to the taste of the liquid contained in the leaves [5].

The plant originated from Sudan. *Aloe* spp. grows in arid climates and its native to Africa, India and other arid areas of the world [6]. Its ability to colonize dry areas stems from the fact that the plant is perennial, drought resistant and succulent [7]. The ability to store vast amounts of water in its thick leaves allows it to survive long periods of desiccation [8].

When the leaf is cut, an orange-yellow fluid drips off. Upon removal of the green cover from the leaf, clear mucilaginous substances persist, which is made of fibres and water [9]. The substance consists of 99.3% water and 0.7% solids [10].

Aloe spp. has been shown to have anti-inflammatory activity, immunostimulatory and cell growth stimulating activity [11]. In addition, extracts from Aloe spp. have antimicrobial, antiviral and antifungal properties [12].

Despite the wide usage of *Aloe* spp. in medicine, there is no much documentation on the quantities of the active ingredients within the plant's extracts [13]. However, previous studies have shown that the plant has anthraquinones, dihydroxyanthraquinones and saponins [8]. A polysaccharide acemannan derived from the whole plant has antimicrobial activity [14].

Aloe spp. contains vitamins A, B, B2, B6, B12, C and E ingredients that make it to be an excellent antioxidants and anti-inflammation [3]. Enzymes amylase, lipase and carboxypeptidase which are present in the plant's extracts help in the breakdown of fats,

sugars and starch. Carboxypeptidase in particular acts as on bradykinin acting against inflammation and controlling vasodilation [15]. This indicates that the plant can be used in remedying hypertension [8].

The current study was conceived in order to extract crude extracts from *Aloe vera, Aloe volkensii* and *Aloe secundiflora*. Further the study aimed at carrying out antimicrobial tests of the extracts against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Erwinia carotovora, Candida albicans* and *Fusarium oxysporum*.

Materials and Methods

The study area

The study was conducted at Egerton University, main campus Njoro in Kenya. Egerton University is located in Njoro Sub County with coordinates as 0° 23' south, 35° 35' and altitude of 2000m above sea level. Temperatures range between 17 - 22°C while the average annual rainfall is 1000 mm [16].

Collection and extraction of *Aloe* spp. extracts

Fresh leaves of *Aloe vera, Aloe volkensii* and *Aloe secundiflora* (Figure 1) were collected from Egerton University around the Department of biological sciences laboratories. Separately, the gel was extracted from the leaves by crushing using a pestle and a mortar. The outer green rinds and leaf pulps were separately dried at 50°C in the oven. The dried parts of the leaves were ground into powder using pestle and mortar. Briefly, 250g of the leaf, green rind powder and 250 ml of gel were separately mixed in 300 ml of methanol and allowed to stand overnight [8]. The mixtures were filtered using Whatman No. 1 filter papers. The extracts were concentrated using a hot air oven at 40°C to dryness.

Figure 1: Photos of Aloe vera, Aloe volkensii and Aloe secundiflora.

Test microorganisms

The antibacterial assay was carried out using *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Erwinia carotovora, Candida albicans* and *Fusarium oxysporum*. The organisms were obtained from the Department of Biological Sciences Egerton University.

Phytochemical screening of the crude extracts

The phytochemical screening of the crude extracts was carried out in order to determine the presence of secondary metabolites such as saponins, alkaloids, flavonoids, steroids, tannins, cardiac glycosides, glycosides, and proteins using standard methods of analyses [17].

Antimicrobial activity of the crude extracts

The antibacterial activity was assessed by agar well assay [8]. The dried extracts were separately re-suspended using 10% methanol to obtain a final concentration 100 mg/ml. The bacterial pathogens were grown overnight in broth to obtain a turbidity equivalent to a turbidity of 0.5 McFarland standard. The number of bacterial concentration at this turbidity is taken to be 1.5 x 10^8 cfu/ml. The cultures were swabbed onto sterile Mueller Hinton agar using sterile cotton swab. About 0.1 ml of each fungus was separately mixed with molten sterile potato dextrose agar. The agar was then poured into sterile Petri-dishes and allowed to cool. Wells were bored into each of the plates using sterile cork borer of 6 mm in diameter. Following this, 0.1 ml of each of the extracts was poured into the wells using a micropipette. The Petri-dishes were placed in refrigerator to allow the extracts to diffuse for 1h. The bacterial pathogens were incubated at 37°C for 24h. The *Candida albicans* and *Fusarium oxysporum* were incubated for 28°C for 2 days and 5 days respectively.

Minimum Inhibitory Concentration (MIC) of the crude extracts

The minimum inhibitory concentrations (MIC) of the extracts were determined by broth tube dilution procedure using two-fold dilution in nutrient broth and sabouraud dextrose broth for bacteria and fungi respectively. In this method *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli, Candida albicans* and *Fusarium oxysporum*.

For each microorganism, 12 sterile screw capped test tubes were used. In each case, a volume of 1 ml nutrient broth was dispensed into test tubes 1 - 10 and 2 ml into test tube 11 (broth control). On the other hand, 1 ml of the crude extract solution was added into test tubes 1 and 2 and 2 ml to test tube 12 (crude extract control). One milliliter of well mixed solution was transferred from test tube 2 to 3 and this process was continued serially up to the test tube 10 by mixing and changing the micropipette tips at each dilution. Finally, 1 ml was discarded from test tube 10 and 0.1 ml of standardized inocula was added into test tubes 1 - 10 and incubated at 37°C for 24h for bacteria and 7 d for fungi. This was repeated for the four antimicrobial extracts. After incubation, by observing the growth of bacteria in the test tube MIC values were determined.

Determination of microbicidal and microbistatic property

The minimum microbicidal and microbistatic concentration of the plant extract against the microbes was determined using the method of [1]. The tubes of the MIC that showed no growth of the microbes were sub-cultured by streaking using sterile wire loop on nutrient agar plates, *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumonia, Escherichia coli* and potato dextrose agar for *Candida albicans* and *Fusarium oxysporum*. The plates were incubated at 37°C for 24 hours (bacteria), 28°C for 2 days (*Candida*

albicans) and 28°C for 5 days (*Fusarium oxysporum*). The minimum microbicidal and microbistatic property was taken as the lowest concentration of the extract that showed no any colony growth on the agar plates.

Results

Phytochemical screening of the extracts

In *Aloe* vera, the extracts showed high levels of saponins in green rid aqueous, glycosides in gel and alkaloids in leaf pulp aqueous (Table 1). There were no sterols in green rid aqueous, leaf pulp aqueous and gel. In addition, there were no proteins in gel. However, there were high presence of proteins (green arid aqueous), glycosides (leaf pulp aqueous) and flavonoids (gel) in *Aloe volkensii*. There was absence of sterols in green rid aqueous, leaf pulp aqueous and gel. Proteins were also missing in gel. However, in *Aloe secundiflora* the highest levels of tannins (green rid aqueous), alkaloids (leaf pulp aqueous) and alkaloids (gel) were witnessed. There was absence of sterols in green rid aqueous, leaf pulp aqueous and gel.

Components	*Aloe vera*			*Aloe volkensii*			*Aloe secundiflora*		
	GRA	**LPA**	**Gel**	**GRA**	**LPA**	**Gel**	**GRA**	**LPA**	**Gel**
Saponins	+++	++	+	++	++	+	++	++	+
Glycosides	+	++	+++	+	+++	++	+	++	++
Flavonoids	+	+	++	+	+	+++	+	+	++
Sterols	-	-	-	-	-	-	-	-	-
Tannins	+	+	+	+	+	+	+++	+	+
Proteins	++	+	-	+++	+	-	++	+	+
Alkaloids	++	+++	++	++	++	++	++	+++	+++

Table 1: Phytochemical screening of extracts from *A. vera, Aloe volkensii* and *Aloe secundiflora*
GRA: Green Rid Aqueous; LPA: Leaf Pulp Aqueous; +++: High; ++: Moderate; +: low; -: Absent

Antimicrobial activity of the crude extracts from *Aloe vera, Aloe volkensii* and *Aloe secundiflora*

In *aloe vera*, the zones of inhibition ranged between *Staphylococcus aureus* (17 ± 2 - 19 ± 2 mm), *Bacillus subtilis* (18 ± 2 - 20 ± 1 mm), *Klebsiella pneumonia (*17 ± 1 - 19 ± 3 mm), *Escherichia coli* (16 ± 1 - 20 ± 3 mm), *Candida albicans* (18 ± 2 - 19 ± 1 mm) *and Fusarium oxysporum* (19 ± 2 - 20 ± 1 mm) (Table 2). However, in *Aloe volkensii*, the ranges were *Staphylococcus aureus* (19 ± 1 - 20 ± 2 mm), *B. subtilis* (17 ± 2 - 21 ± 3 mm), *Klebsiella pneumoniae* (18 ± 2 - 19 ± 1 mm), *Escherichia coli* (18 ± 2 - 19 ± 3 mm), *Candida albicans* (17 ± 1 - 20 ± 1 mm) and *Fusarium oxysporum* (19 ± 2 - 21 ± 1 mm). In addition, the zones of inhibition in *Aloe secundiflora* varied from *Staphylococcus aureus* (17 ± 1 - 20 ± 1 mm), *Bacillus subtilis* (18 ± 2 - 19 ± 2 mm), *Klebsiella pneumoniae* (17 ± 3 - 20 ± 2 mm), *Escherichia coli (*19 ± 1 - 20 ± 2 mm), *Candida albicans (*17 ± 1 - 19 ± 1 mm) and *Fusarium oxysporum* (17 ± 2 - 19 ± 1 mm). The zones of inhibition varied significantly (F = 3.424051, P = 0.005007). among *Aloe vera*, *Aloe volkensii* and *Aloe secundiflora*.

Minimum inhibitory concentration of the *Aloe vera, Aloe volkensii* and *Aloe secundiflora* crude extracts against test microorganisms

The minimum inhibitory concentration in *Aloe vera* ranged between *Staphylococcus aureus* (0.40 ± 1 - 10.45 ± 1 mg/ml), *Bacillus subtilis* (0.41 ± 3 - 0.46 ± 2 mg/ml)*, Klebsiella pneumonia* (0.31 ± 2 - 0.43 ± 3 mg/ml), *Escherichia coli* (0.20 ± 2 - 0.45 ± 2 mg/ml), *Candida albicans* (0.31 ± 1 - 0.40 ± 1 mg/ml) and *Fusarium oxysporum* (0.32 ± 1 - 0.43 ± 1 mg/ml) (Table 3). In *Aloe volkensii,* the ranges were *Staphylococcus aureus* (0.40 ± 1 - 0.44 ± 2 mg/ml), *B. subtilis* (0.42 ± 2 - 0.45 ± 3 mg/ml), *Klebsiella pneumoniae* (0.32 ± 2 - 45 ± 2 mg/ml), *Escherichia coli* (0.25 ± 1 - 0.40 ± 1 mg/ml), *Candida albicans* (0.32 ± 1 - 0.35 ± 1 mg/ml) and *Fusarium oxysporum* (0.34 ± 2 - 0.40 ± 2 mg/ml). In addition, the zones of inhibition in *Aloe secundiflora* varied from *Staphylococcus aureus* (0.39 ± 2 - 0.46 ± 2 mg/ml), *Bacillus subtilis (*0.43 ± 1 - 0.47 ± 1 mg/ml), *Klebsiella pneumoniae (*0.35 ± 2 - 0.48 ± 2 mg/ml), *Escherichia coli (*0.25 ± 1 - 0.45 ± 2 mg/ml), *Candida albicans (*0.34 ± 1 - 0.46 ± 1 mg/ml) and *Fusarium oxysporum* (0.35 ± 2 - 0.48 ± 2 mg/ml). The MICS, MBC'S and MFC were equal. The MICS, MBC and MFC varied significantly (F = 4.696456, P = 0.000534) among *Aloe vera*, *Aloe volkensii* and *Aloe secundiflora.*

Pathogens	*Aloe vera*			*Aloe volkensii*			*Aloe secundiflora*		
	GRA	**LPA**	**Gel**	**GRA**	**LPA**	**Gel**	**GRA**	**LPA**	**Gel**
S. aureus	19 ± 2	18 ± 1	17 ± 2	19 ± 1	20 ± 2	19 ± 1	20 ± 1	19 ± 2	17 ± 1
B. subtilis	20 ± 1	18 ± 2	18 ± 3	20 ± 1	21 ± 3	17 ± 2	19 ± 2	18 ± 3	18 ± 2
K. pneumoniae	19 ± 3	19 ± 1	17 ± 1	18 ± 2	19 ± 1	18 ± 3	19 ± 3	20 ± 2	17 ± 3
E. coli	18 ± 2	20 ± 3	16 ± 1	19 ± 3	18 ± 2	19 ± 1	20 ± 2	19 ± 1	19 ± 1
C. albicans	19 ± 1	19 ± 1	18 ± 2	20 ± 1	20 ± 1	17 ± 1	19 ± 1	18 ± 1	17 ± 1
F. oxysporum	20 ± 1	19 ± 2	19 ± 3	19 ± 2	21 ± 1	19 ± 2	18 ± 1	19 ± 2	17 ± 2

Table 2: Zones of inhibition in mm of the selected microorganism against crude extracts of *Aloe vera, Aloe volkensii* and *Aloe secundiflora*.
Each value represents the means (± SD) of five independent experiments, *S. aureus: Staphylococcus aureus; B. subtilis: Bacillus subtilis; K. pneumonia: Klebsiella pneumonia; E. coli: Escherichia coli; C. albicans: Candida albicans; F. oxysporum: Fusarium oxysporum*; GRA: Green Rid Aqueous and LPA: Leaf Pulp Aqueous.

Microorganism	*Aloe vera*			*Aloe volkensii*			*Aloe secundiflora*		
	GRA	LPA	Gel	GRA	LPA	Gel	GRA	LPA	Gel
S. aureus	0.40 ± 1	0.42 ± 2	0.45 ± 1	0.40 ± 1	0.41 ± 2	0.44 ± 2	0.39 ± 2	0.43 ± 1	0.46 ± 2
B. subtilis	0.41 ± 3	0.43 ± 1	0.46 ± 2	0.42 ± 2	0.46 ± 1	0.45 ± 3	0.43 ± 1	0.42 ± 2	0.47 ± 1
K. pneumoniae	0.31 ± 2	0.35 ± 2	0.43 ± 3	0.32 ± 2	0.36 ± 2	0.45 ± 2	0.35 ± 2	0.40 ± 2	0.48 ± 2
E. coli	0.20 ± 2	0.25 ± 1	0.45 ± 2	0.25 ± 1	0.30 ± 1	0.40 ± 1	0.25 ± 1	0.30 ± 2	0.45 ± 2
C. albicans	0.31 ± 1	0.35 ± 2	0.40 ± 1	0.32 ± 1	0.34 ± 1	0.35 ± 1	0.34 ± 1	0.35 ± 1	0.46 ± 1
F. oxysporum	0.32 ± 1	0.37 ± 2	0.43 ± 1	0.34 ± 2	0.35 ± 3	0.40 ± 2	0.35 ± 2	0.36 ± 2	0.48 ± 2

Table 3: Minimum inhibitory concentration of the *Aloe vera, Aloe volkensii* and *Aloe secundiflora* crude extracts against test microorganisms.

Each value represents the means (± SD) of five independent experiments, *S. aureus: Staphylococcus aureus; B. subtilis: Bacillus subtilis; K. pneumonia: Klebsiella pneumonia; E. coli: Escherichia coli; C. albicans: Candida albicans; F. oxysporum: Fusarium oxysporum*; GRA: Green Rid Aqueous and LPA: Leaf Pulp Aqueous.

Discussion

The results of the current study on phytochemical compounds present in the extracts of Aloe vera, Aloe volkensii and Aloe secundiflora partly agrees with a previous study carried out in India [11]. The possible cause of the similarity may ne similarities in varieties of the Aloe sp. studied [18] maintained that same varieties of aloe are likely to produce the same secondary metabolites. However [19] says that the environment an Aloe spp. grows in influences the metabolites that it produces.

In a study on comparative study of antimicrobial activity of *Aloe vera* gel and antibiotics against isolates from fast food [20] obtained lower zones of inhibition than in the current study. This may have emanated from the type of secondary metabolites synthesized by the *Aloe* sp. under study [21]. However [22] obtained zones of inhibition that concurred with those of the current study [23] explains that use of the same extraction procedures lead to extraction of the same metabolites.

However the results of the present study on minimum inhibitory concentration, minimum bactericidal concentration and minimum fungicidal concentration differ with a previous study carried out elsewhere [24]. This may have been caused by differences in the sources of the pathogens being studied [25]. Further [26] asserts that though strains of pathogens may be the same, their habitat may lead to mutations which influences their response to varied crude extracts.

Conclusion

Three varieties of *Aloe* spp. namely *Aloe vera*, *Aloe volkensii* and *Aloe secundiflora* were collected from the Department of Biological Sciences, Egerton University. Different crude extracts from them were obtained having varying secondary metabolites. The antimicrobial properties of the crude extracts were tested against *Staphylococcus aureus, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Candida albicans* and *Fusarium oxysporum*. The minimum inhibitory concentration was equal to minimum bactericidal concentration and minimum fungicidal concentration suggesting that the crude extracts were bactericidal and fungicidal.

Recommendations

There is need to extract the secondary metabolites from Aloe vera, Aloe volkensii and Aloe secundiflora in large scale. Structure elucidation of the active metabolites from the metabolites need to be carried out.

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Conflict of Interest

The authors declare no conflict of interest.

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