Pak. J. Sci. Ind. Res. 2007 **50**(2) 124-127

Antibacterial Property of *Aloe vera* Plant Extract

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(received May 8, 2006; revised April 6, 2007; accepted April 10, 2007)

Abstract. The antibacterial activity of *Aloe vera* (*barbadensis* Miller) was investigated on some test organisms using the agar diffusion and tube dilution methods. Ethanol, hot water and distilled water were the solvents used for extraction of the active ingredients from the plant leaf. The test organisms used were *Streptococcus pyogenes*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus* sp., *Klebsiella* sp., and *Escherichia coli*. The results showed that *Aloe vera* ethanolic extract had more antibacterial activity than the hot water and distilled water extracts producing the largest zone of inhibition (22 mm) on *S. aureus*. The results also showed that all the organisms tested were susceptible to the extracts except *E. coli*, which was resistant to the cold distilled water extract. For susceptible strains, the diameter of zones of inhibition ranged from 9 mm - 22 mm. The results of the susceptibility tests showed that the *A. vera* plant extracts compared favourably with tested commercial antibiotics. The minimum inhibitory concentration (MIC) results revealed that the plant extracts had low MIC values, ranging from 0.15625-0.625 μg/ml.

Keywords: *Aloe vera*, extract, antibacterial property

Introduction

Aloe vera is a perennial drought resisting and succulent plant belonging to the family, Liliaceae (Miller and Koltain, 1995). The true *A. vera* plant is called *A. barbadensis* Miller otherwise called the curacaro aloe. Essentially, two products are obtained from the *A. vera* leaves the clear gel and the yellowish sap, which is very bitter (Bloomfield, 1985). The clear gel is used to treat skin irritations while the sap is used as a laxative (Grindley and Reynolds, 1986). *A. vera* gel comprises more than seventy five compounds, including polysaccharides, steroids, organic acids, enzymes and a lkaloids. The gel also contains 99.3% water and the remaining 0.7% is solids (Hegger *et al.*, 1996).

Early medical tests from India indicated that the *Aloe vera* was already being used in the fourth century for skin inflammation (Bloomfield, 1985). The clear gel of *A. vera* leaf has both anti-tumour and chemopreventive effects. Both effects are caused by stimulation of the immune system and the strong antioxidants properties of the gel itself (Pengs *et al.*, 1991). Davis *et al.* (1994) devised an experiment to examine the wound healing capability of *A. vera* in both, oral and topical forms. The results showed that *Aloe* plants improves the healing of wounds, leads to better vascularity, and heal their granulation of tissues. According to Schulz and Schynder (1993), *Aloe vera* is an additive to shampoos, and moisturizers, it strengthens the hair and cares for the scalp, preventing hair breaking and splitting. Reports suggest that the beneficial effects of *Aloe vera* gel are due to its high molecular weight compounds such as polysaccharides (Egger *et al.*, 1996; Shida *et al.*, 1985), lectin like proteins (Grindley and Reynolds, 1986) and prostagladins (Azfal *et al.*, 1991). Due to medicinal importance of *A. vera* plant and its use in formulation of new pharmaceutical products, the present study was carried on the antibacterial property of *Aloe vera* extracts.

Materials and Methods

Pure cultures of test organisms (*Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes, Escherichia coli, Bacillus* sp. and *Klebsiella* sp.) were obtained from Otibhor Okhae Teaching Hospital Laboratory in Irrua, Edo State, Nigeria. *S. aureus* NCTC 6571 and *E. coli* NCTC 10414 were used as control strains. The identity of the organisms were confirmed using various morphological and biochemical tests, according to Barrow and Feltham (1993).

Aloe vera plants were obtained from Eguare in Ekpoma. The leaves were identified as *Aloe vera* (*barbadensis* Miller) at the Botany Department, Ambrose Alli University, Ekpoma.

Extraction of crude extract of *Aloe vera* leaves. The active compounds of *Aloe* leaves were extracted according to the

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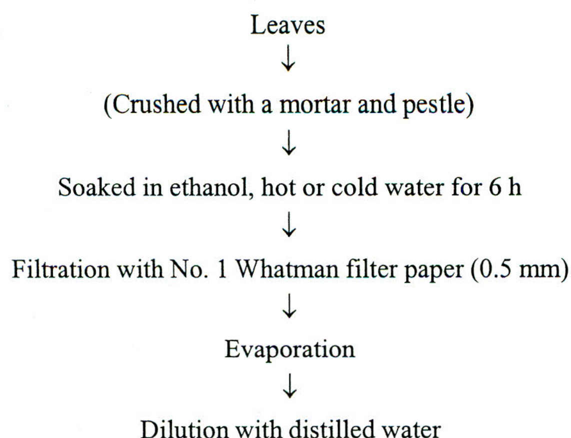
procedure described by Coats (1979). A schematic representation is illustrated as follows:



The leaves were thoroughly washed in distilled water and dried with the hot air oven at 60 °C for 3 days. The amount of water loss was calculated by subtracting the weight of the fresh leaves from the dry leaves.

The dried leaves were pounded with the mortar and pestle and 5 g each of the sample was placed into three separate 250 ml beakers. In the first beaker, 50 ml of hot water was poured, the second had 50 ml of ethanol while in the third beaker, 50 ml of cold distilled water was added. The beakers were then covered with aluminum foil and allowed to stand for 5-7 h with occasional stirring. The contents were filtered by passing the suspension through sterile Whatman filter paper disc # 1 (0.5 mm), the ethanol filtrate was evaporated after filtration. Double dilution of the gel was prepared by diluting against distilled water.

Determination of antimicrobial activity of *Aloe vera* (*barbadensis* Miller) plant extract. The test organisms and the standard strains were separately inoculated into nutrient broth and incubated at 37 °C for 4-6 h. Then 0.2 ml (10^5 cfu/ml) of the broth culture of the test bacteria were seeded on Mueller-Hinton agar plates and spread evenly.

Six wells of 6 mm in diameter were cut on the seeded agar plates using a sterile cork borer. Three of the wells were each filled with 0.05 ml of cold, hot water extracts and ethanol extract.

The remaining three wells contained cold, hot water and ethanol each and these served as control wells. The plates were incubated at 37 °C for 24 h after which the zones of inhibition round the wells determined the antibacterial activity of the extracts. Zone diameter was recorded as the differences between extracts and any produced by the respective controls. Seasonal patterns of the test organisms to conventional antibiotics were also determined using disc diffusion method and zones of inhibition, were compared with those of the extracts. The results were read according to the method of Stokes and Ridway (1980), which states that (a) "Sensitive" if the zone radius is equal to or not more than 3 mm less than the control. (b) "Intermediate" if the zone radius is more than 4 mm smaller or less than the control but not less than 3 mm. (c) "Resistant" if the zone radius is 2 mm or less (negative) or no zone of inhibition.

Determining the minimum inhibitory concentration (MIC). The method of Stokes and Ridway (1980) was used to determine the MIC of the plant extract on the test organisms. A total of 8 test tubes were arranged on a test tube rack. Thereafter, 1 g of the plant extract was added into the first test tube containing 10 ml of distilled water, and agitated gently. From the first test tube, 1ml of the dilution was transferred into the 2nd test tube upto the 7th test tube that contained 1 ml of distilled water each. Test tube 8 had no extract and it served as control.

An aliquot (0.2 ml, 10^5 cfu/ml) of the broth culture containing the test organisms was added to each tube. The set up was incubated overnight and observed for growth.

The MIC was recorded as the lowest concentration (highest dilution) of the extract that inhibited visible growth. The initial concentration of the extracts was determined by the gravimetric method as determined by Orhue, (2003), stated below:

i. the weight of 50 ml specific gravity (S.G.) bottle with the solvent was taken with the aid of mettler balance (W_1);
ii. the extracted material and the solvent were filled into sterilized S.G bottle and the weight taken (W_2);
iii. dry weight of extract W_2 - W_1 ------- (W_3)
iv. concentration of extract per ml is W_3 divided by 50 (capacity of SG bottle).

Results and Discussion

The results obtained from the antimicrobial activity assay, show that *Aloe vera* extract has antibacterial activity against the six (6) clinical isolates used in this study.

The initial (preliminary) screening by the agar diffusion method showed that *S. aureus*, *Bacillus* sp., *S. pyogenes*, *P. aeruginosa*, *Klebsiella* sp., and *E. coli* were susceptible to the extracts, and gave zone inhibition diameters of 22 mm, 16 mm, 15 mm, 19 mm, 14 mm and 10 mm, respectively, for ethanol extract of fresh leaves (Table 1).

The hot water extract produced zones of inhibition of 17 mm, 13 mm, 11 mm, 15 mm, 12 mm and 8 mm for

S. aureus, *Bacillus* sp., *Streptococcus pyogenes*, *P. aeruginosa*, *Klebsiella* sp., and *E. coli,* respectively (Table1). For the distilled water extract, *Aloe vera* had antibacterial activity against the organisms tested except *E. coli* (Table 1). For all extracts, the inhibitory effect was relatively more pronounced on *S. aureus* (14 mm) among the tested bacterial species (Table 1), while zone of inhibition of 12 mm, 9 mm, 13 mm and 11 mm were produced by *P. aeruginosa*, *Bacillus* sp., *S. pyogenes,* and *Klebsiella* sp., respectively.

The initial concentration of the extracts was 0.6 μg/ml, 0.56 μg/ml and 0.46 μg/ml for ethanol, hot water and cold distilled water, respectively. For the ethanolic extract the MIC range was 0.625-0.15625 μg/ml for all the test isolates except *E. coli*, which was 0.3125 μg/ml.

For the hot water extract, the MIC values range were 0.562-0.15625 μg/ml; the MIC was 0.5625 μg/ml for *S. aureus, S. pyogenes*, *Klebsiella* sp., and *P. aeruginosa* while *Bacillus* sp. had an MIC of 1.125 μg/ml and *E. coli* was 0.28125 μg/ml. The distilled water extract produced MIC value of 0.5 μg/ml for *S. aureus, Bacillus* sp. and *S. pyogenes* and 0.25 μg/ml for *Klebsiella* sp., *P. aeruginosa* and *E. coli. In vitro* antibiotic susceptibility test carried out on the isolates showed that 16 (66.7%) were sensitive to ofloxacillin (10 μg), 15 (62.5%) to cloxacillin (5 μg), 14 (58.3%) to gentamycin (10 μg), 8 (33.3%) to both erythromycin (5 μg) and tetracycline (10 μg), 7 (29.3%) to ciprofloxacin (10 μg), 6 (25 μg) to ceflazidine (5 μg), 5 (20.8%) to both colistin (10 μg) and cotrimoxazole (25 μg), 4(16.7%) to clindamycin (20 μg), 3(12.5%) to streptomycin (20 μg) and 1(4.2%) to ampicillin (10 μg). Penicillin did not inhibit growth of any of the isolates (Table 2).

Results from this study showed that ethanolic extract of the leaves of *Aloe vera* has more antibacterial activity followed by hot water and distilled water extracts on all the test organisms. This suggests that ethanol and hot water extracted the most active components of the plant. It also showed that of all the organisms tested, *S. aureus* was more susceptible to ethanolic, hot water and distilled water extracts with inhibitory zones ranging from 14-22 mm.

Usually, the degree of susceptibility is often expressed quantitatively as the lowest concentration of a drug or extract from a plant that inhibits the growth of a given strain. This is usually expressed as the minimum inhibitory concentration (MIC) and it varies with the concentration of the antimicrobial substance. The MIC results showed that the plant extract had low

Table 1. Antimicrobial activity of *Aloe vera* leaf extracts

Organisms	Zone diameter of inhibition (mm)		
	Ethanol	Hot water	Cold water
Staphylococcus aureus	22	17	14
Pseudomonas aeruginosa	19	15	12
Bacillus sp.	16	13	9
Streptococcus pyogenes	15	11	13
Klebsiella sp.	14	12	11
Escherichia coli	10	8	-

Table 2. Antibiotic susceptibility test of the isolates

Antibiotics	Test organisms							
	S. aureus (N = 8)	*E. coli* (N = 6)	*Klebsiella* sp. (N = 4)	*Bacillus* sp. (N = 3)	*P. aeruginosa* (N = 2)	*S. pyogenes* (N = 1)	Frequency (N = 24)	Rate of sensitive strains in %
Ofloxacin (10 μg)	8	-	4	2	2	-	16	66.7
Cloxacillin (5 μg)	8	-	3	1	2	1	15	62.5
Gentamycin (10 μg)	6	3	3	-	2	-	14	58.3
Erythromycin (5 μg)	7	-	1	-	-	-	8	33.3
Tetracycline (10 μg)	5	-	3	-	-	-	8	33.3
Ciproxin (10 μg)	3	-	1	-	-	1	7	29.2
Ceflazidine (5 μg)	-	4	-	1	-	1	6	25.0
Colistin (10 μg)	-	-	3	-	2	-	5	20.8
Cotrimoxazole (25 μg)	2	3	-	-	-	-	5	20.8
Clindamycin (20 μg)	4	-	-	-	-	-	4	16.7
Streptomycin (20 μg)	3	-	-	-	-	-	3	12.5
Ampicillin (10 μg)	-	-	1	-	-	-	1	4.2

N = number of isolates

MIC concentration and the sensitivity of the test organisms to the plant extract compares favourably with commercial antibiotics, putting the initial concentration of the plant extract into consideration (Table 2).

This implies that the extract could be effectively used in the treatment of some infections or diseases caused by the various test organisms. For instance, *S. aureus* was resistant to ampicillin but susceptible to *Aloe vera* leaf extract. High rates of sensitivity of bacterial isolates were recorded with ofloxacin (66.7%) cloxacillin (62.5%) and gentamycin (58.3%).

These results were similar to those reported by Davis *et al.* (1994) that among the healing properties of *A. vera*, one of the most promising for medicine is its use in wound healing. With the ethanolic extract producing zone diameters of inhibition of 22 mm and 19 mm on *S. aureus* and *P. aeruginosa,* respectively, this extract could be employed in the treatment of wound infections due to these bacterial isolates. Although *in vitro* zones of inhibition observed with one drug cannot be compared with those obtained with another antimicrobial agent due to the differences in rate of diffusion through agar gel amongst other factors (Barry and Thornsberry, 1993), the zones observed for *Aloe vera* extract compares favourably with those of standard zone for known organisms.

Since the zones of inhibition of hot water extracts compares favourably with those of ethanolic extract, patients or individuals who cannot withstand alcohol could use hot water extracts of these plants.

The results of this study confirm why *Aloe vera* gel is used as a common additive to shampoos, moisturizers and soaps as earlier reported by Schulz and Schynder (1993). It is believed that the antibacterial constituents of this plant leaf when extracted and purified will be a useful formulation in the treatment of some bacterial infections.

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