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Antibacterial Activities of Crude Extract of *Aloe barbadensis* to Clinically Isolated Bacterial Pathogens

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Abstract The antibacterial activity of *Aloe barbadensis* was tested on clinically isolated bacterial pathogens i.e. *Enterococcus bovis, Staphylococcus aureus, Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Morganella morganii*, and *Klebsiella pneumoniae* causing infection in human being. Ethanolic and aqueous extracts were used for the antibacterial effect, which was measured by the appearance of zone of inhibition. Relatively higher MIC concentrations were obtained for gram negative bacteria *E. coli* and *K. pneumoniae*, with ethanol extract; however, no inhibitory effect was noted for aqueous extract. Ethanolic extract possesses great inhibitory activity for gram positive bacteria, *E. bovis* followed by *S. aureus*. Among gram negative bacteria, highest inhibitory effect was observed with *P. aeruginosa*, followed by *M. morganii*, *P. mirabilis*, and *P. vulgaris*, which was significant ($p<0.01$) than *E. coli* and *K. pneumoniae*. Antimicrobial activity tests of crude extract of *A. barbadensis* were carried out to validate the use of traditional medicinal herbal and results of this study tend to give credence to the common use of *A. barbadensis* gel and leaf.

Keywords *Aloe vera* · Antibacterial · Ethanolic extract · Herbal · Medicinal plant

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Introduction

Multi-drug resistance is a world-wide problem, attributed to the extensive use of antibiotics, selection pressure on bacterial strains, and lack of new drugs, vaccines, and diagnostic aids. These shortcomings lead to an urgent global call for new antimicrobial drugs, particularly from natural resources [1]. Majority of medicinal plant species are rich in biomolecule contents which can cope with health hazard and recently, antibacterial activity of many plant species have been reported [2–4]. Among these medicinal plants, *Aloe vera* or *Aloe barbadensis* has been used therapeutically for centuries and is of particular interest due to lengthy historic reputation as a curative agent and its widespread use in complementary therapies. Aloe is a well-known natural dietary supplement and chemopreventive agent. Aloe gel has been used for a long time for topical treatment of skin irritations, but aloe can also be used as a beverage and aloe products are considered to provide relief from constipation. Aloe extract can be taken orally as a dietary supplement, even though it does not have FDA approval to be used as a drug [5]. *A. vera* gel is the leaf pulp of mucilage, a thin clear jelly-like substance obtained from the parenchymal tissue that makes the inner portion of the leaves [6].

The gel consists of 99.3% water and remaining 0.7% is made up of solids with glucose and mannose constituting a large part. The active components of *A. barbadensis* include aloesin (2-acetonyl-8-β-D-glucopyranosyl-7-hydroxy-5-methylchromone), anthraquinones (aloin and aloe emodin), aloemannan, acemannan (gel polysaccharides), aloeride, verectin, giberellin-like substance, aloeresin I, 5-methylchromone, flavonoids, glycoprotein fraction, G1G1M1DI2, anthraglycosides, reducing sugars, cardiotonic glycosides, saponins, naftoquinones, sterols, triterpenoids [7], amino acids, and vitamins [8]. These compound are the source of polysaccharides and possess antibacterial, antiviral, antifungal, antioxidant, angiogenic, immune system modulator, antidiabetic, antihypertensive, cathartics, analgesic, anti-inflammatory, wound healing, antihepatitis, antigastric ulcer, and antineoplastic activities [7, 9].

Nevertheless, very few studies have been carried out on leaf extract of Aloe and most of them have been conducted on gel [1]. In this study, we determine the antimicrobial activities of *A. barbadensis* leaf extract. Although a lot of work has been carried out on the medicinal applications of *A. barbadensis* gel, there is still little information on the uses of the leaf [10]. This work therefore provides information on the antibacterial activity (against the microorganisms causing skin, upper respiratory tract, gastrointestinal, and urinogenital tract infection) of leaf extract of *Aloe barbadensis.*

Material and Methods

Test Organisms

Pure bacterial cultures (Table 1) were clinically isolated from patients by the Department of Medical Microbiology, Uttarakhand Forest Hospital Trust Medical College, Haldwani, India.

Extraction from *A. Barbadensis*

A. barbadensis Mill leaf latex (4.5 kg after removing the epidermis) was cut into small pieces and homogenized. The homogenized plant material was extracted with ethanol (95%) and sterile MiliQ water. The extracts (aqueous and ethanol) were evaporated at 45°C temperature under reduced pressure to a syrup like residue. The solvent was completely

Table 1 List of bacterial pathogens isolated from patients.

Bacteria	Diseases
Enterococcus bovis	Urinary tract infection (UTI)
Staphylococcus aureus	UTI, upper and lower respiratory tract infection, staphylococcal scalded skin syndrome (SSSS), septic arthritis, staphylococcal endocarditis (infection of the heart valves), pneumonia, skin infections (may occur as a commensal on human skin; it also occurs in nose frequently) such as pimple sand impetigo, meningitis, toxic shock syndrome (TSS)
E. coli	UTI, cystitis and acute pyelonephritis
Proteus vulgaris	UTI, wound infections, respiratory infections
Proteus mirabilis	Urinary tract infection (UTI)
Pseudomonas aeruginosa	UTI, upper and lower respiratory tract infection
Klebsiella pneumoniae	UTI, pneumonia, it is an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. It is more commonly implicated in hospital-acquired urinary tract and wound infections, particularly in immunocompromised individuals
Morganella morganii	Urinary tract infection (UTI)

removed and dried ethanol and aqueous extracts were re-dissolved in different concentrations in their respective solvents (ethanol and MiliQ water) to determine MIC.

Determine Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) was determined by the dilution method as recommended by the National Committee for Clinical Laboratory Standards [11]. Different concentrations of ethanolic and aqueous *A. barbadensis* leaf extracts (ranging from 25 mg/ml to 0.1 mg/ml) were tested separately for each bacterium and inhibition zone of microbial growth in the plates containing tested solutions was judged by comparison with blank control plates. Minimum inhibitory concentration is defined as the lowest concentration of test samples that result in a complete inhibition of visible growth. Experiments were carried out in triplicate.

Antibacterial Activities

The antibacterial susceptibility tests were carried out using agar diffusion method [12], which is routinely used in hospitals to test antimicrobial susceptibility for antibiotic-resistant bacteria, followed by the dilution method for products which possess a bioactivity [13, 14]. Leaf extracts (MIC of each bacterium) were delivered into well and plates were incubated at 37 °C for 24 h. The presence of zone of inhibition was regarded as the presence of antimicrobial action and antimicrobial activity was expressed in terms of average diameter of the zone of inhibition measured in millimeter. Each test was carried out in triplicate.

Statistical Analysis

Analysis of variance was used to analyze data and determine differences [15]. Data was expressed as mean±SE. A Tukey HSD multiple comparison of mean test was used when significant differences were found and $p<0.01$ was considered as significant.

Table 2 Antibacterial activity of *Aloe barbadensis* extract.

Type of bacteria	Bacteria	Zone of inhibition[a] (mm)		Minimum inhibitory concentration (MIC) for ethanolic extract (mg/ml)
		Ethanolic extract (mean±SE)	Aqueous extract (mean[b])	
Gram Positive	*Enterococcus bovis*	30.0±3.21	4.0	0.50
	Staphylococcus aureus	20.67±0.67	3.0	0.50
Gram Negative	*Escherichia coli*	9.67±0.33	3.0	10.0
	Proteus vulgaris	17.67±0.33	4.0	0.50
	Proteus mirabilis	19.33±0.33	4.0	0.50
	Pseudomonas aeruginosa	26.33±1.33	4.0	0.10
	Klebsiella pneumoniae	8.0±1.0	4.0	10.0
	Morganella morganii	24.0±1.0	4.0	0.30

[a] Excluding the diameter of wells

[b] Statistical analysis was not implemented

Results and Discussion

Leaf extract of *A. barbadensis* (both aqueous and ethanolic) tend to inhibit gram positive bacteria, *Enterococcus bovis* and *Staphylococcus aureus*. However the inhibitory activity was very low in aqueous extract (3–4 mm, Table 2) in comparison to ethanolic extract (20–30±SE mm). Same pattern was also observed with gram negative bacteria, *Escherichia coli, Proteus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Morganella morganii*,

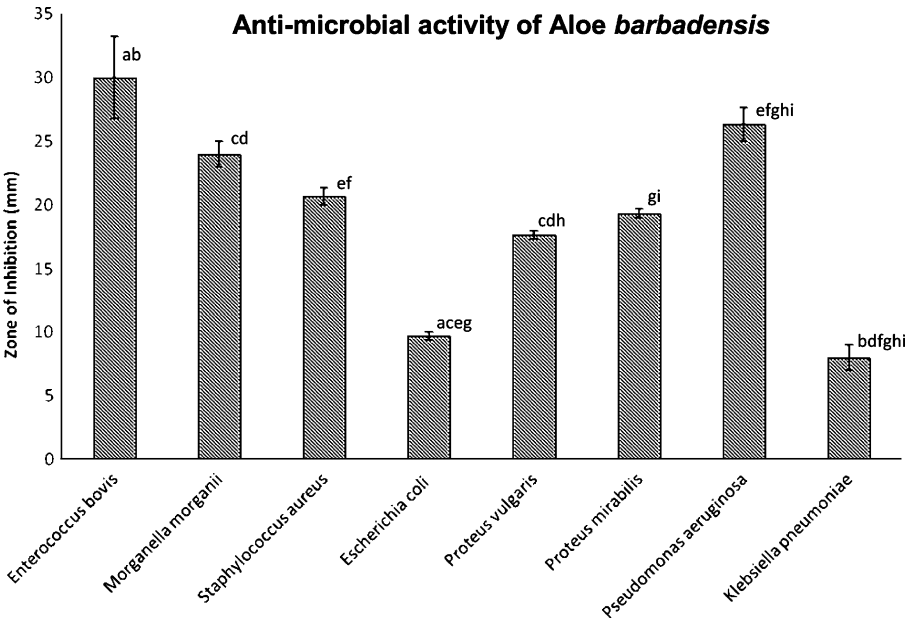

Fig. 1 Effect of ethanolic extract of *Aloe barbadensis* on different clinically isolated bacterial pathogens. *Error bars* indicate ±SE and *similar lower case letters* indicate significant difference ($p<0.01$)

and *Klebsiella pneumoniae* (Table 2). Relatively higher MIC concentrations were obtained for gram negative bacteria *E. coli* and *K. pneumoniae* (Table 2) with ethanol extract. Surprisingly, no inhibitory effect has been noted for aqueous extract, this could be attributed to the extraction of active component of *A. barbadensis* in ethanol rather than water. Coopoosamy and Magwa [14] also observed activity in acetone extract only while using crude extract of *Aloe excelsa*.

Results show that ethanolic extracts possess great inhibitory effect for gram positive bacteria, *E. bovis* followed by *S. aureus* (Fig. 1). Among gram negative bacteria highest inhibitory effect was observed with *P. aeruginosa*, followed by *M. morganii*, *P. mirabilis*, and *P. vulgaris*, which was significant ($p<0.01$) than *E. coli* and *K. pneumoniae* (Fig. 1). This result could be responsible for the popular use of *A. barbadensis* gel and leaf to relieve different types of gastrointestinal irritations, skin irritations, upper respiratory tract, intestinal tract, and urinogenital infections [5, 7]. The gel is also known to promote wound healing due to the presence of active components like anthraquinones and chromones [16].

The results are in line with those from previous screenings of medicinal plants for antibacterial activity, where most of the plants showed activity against gram positive strains only [17]. In this manuscript, it has been reported that ethanolic extract of *A. barbadensis* leaf has high antibacterial activity for gram negative as well as gram positive bacteria with a very low MIC.

Conclusion

The results of the viability assay have proved *A. barbadensis* to hold excellent potential as an antibacterial agent. *A. barbadensis* has acemannan (acylated mannose) which makes a mucilaginous layer around the urinogenital, gastrointestinal, and respiratory tract when consumed orally. The layers trap the microbial flora and make them unable to invade the system. *A. barbadensis* has anthraquinones as an active compound, which is structural analogue of tetracycline. The anthraquinones act like tetracycline and inhibit bacterial protein synthesis by blocking the ribosomal A (where the aminoacylated t-RNA enters) site. Therefore, the bacteria can not grow in the media containing *A. barbadensis* extract. Acemannan and anthraquinones both work together in-vivo, while in-vitro, only anthraquinones are effective. Anthraquinones are alcohol, acetone, etc. soluble but poorly or insoluble in water therefore we observed significant antibacterial effect with ethanolic extract. Some bacteria exhibit least sensitivity (as the zone of inhibition is much less in comparison to others) in in vitro conditions, but if the gel or whole leaf or *A. barbadensis* juice is taken orally both acemannan and anthraquinones will work simultaneously and it may be more effective.

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