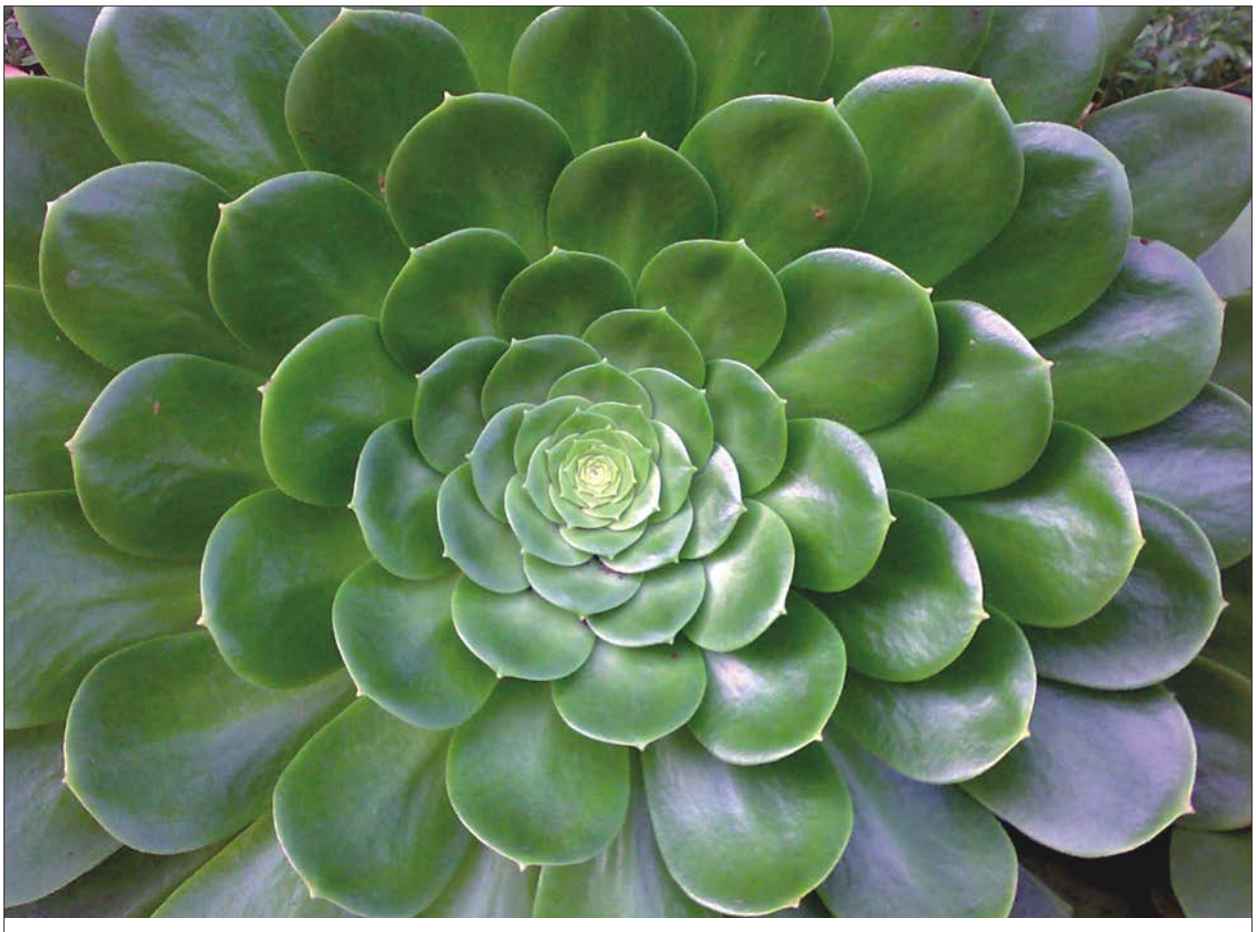

Journal of
Plant Sciences

ISSN 1816-4951

Academic
Journals Inc.

www.academicjournals.com

Journal of Plant Sciences 1 (4): 348-355, 2006
ISSN 1816-4951

In vitro Antibacterial and Antifungal Activities of Ethanolic Extract of *Aloe vera* Leaf Gel

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Abstract: *Aloe vera* leaf gel is widely used as a traditional folk medicine for the treatment of different infectious diseases. *In vitro* antimicrobial properties of ethanolic extract of *Aloe vera* leaf gel were investigated against various common pathogenic bacteria and fungi. The well and disc-diffusion method showed significant zone of inhibition against all the pathogens studied and the results are comparable to the conventional antibiotics. These results support the ethanomedicinal use of *Aloe vera* for the treatment of various ailments.

Key words: *Aloe vera*, antibacterial, antifungal, *In vitro*, antibiotics microorganisms

Introduction

Diseases due to pathogenic bacteria and fungi represent a critical problem to human health and they are one of the main causes of morbidity and mortality worldwide (WHO, 1998). Resistance to antibiotics and with the toxicity during prolonged treatment with present day drugs have been the reasons for an extended search for newer drugs to treat opportunistic microbial infections (Fostel and Lartey, 2000). During this process, the investigation of the efficacy of plant-based drugs in traditional medicine has been paid great attention because these drugs elicit few side effects, cheap and easily available; according to the World Health Organization, 80% of the world population still relies mainly on plant drugs (Kumara, 2001).

Further, most of our marketed medicines are distillations, combinations, reproductions or variations of substances, which are abundantly found in nature. Our fore fathers recommended some of the substances, which are abundantly found in nature long before their value was demonstrated and understood by scientific methods. We should not dismiss any of our common heritages of knowledge unless and otherwise proved negative by the methods of scientific validation.

Aloe vera was known to herbalists and medical folklorists for centuries as the medicinal plant or the Potted physician. Aloe is also mentioned in the Bible several times, for example, in St. John's Gospel that it was used for incense as well a major ingredient used in embalming the body of JESUS CHRIST. The ancient Egyptians referred to Aloe as the plant of immortality and included it among the funerary gifts buried with the pharaohs. Aloe is a cactus-like plant with green dagger shaped leaves filled with a clear viscous gel. The parenchymatous cells in the leaf pulp of *Aloe vera* leaf, commonly known as *Aloe vera* gel, has been used since early times for a host of curative purposes in humans and animals (Grindlay and Reynolds, 1986; Haller, 1990).

Previous studies conducted by us revealed the presence of many biologically active phytochemicals in the ethanolic extract of *Aloe vera* gel (Rajasekaran *et al*., 2005a) and which may be responsible for its hypoglycemic and anti-oxidant properties (Rajasekaran *et al*., 2004, 2005b).

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The present study was conducted to investigate the antimicrobial properties of ethanolic extract of *Aloe vera* gel against *in vitro* gram (+)ve, gram (-)ve and common pathogenic fungi.

Materials and Methods

Plant Material

Mature, healthy and fresh leaves of *Aloe vera* having a length of approximately 2-3 feet were washed with hot (40°C) water, dissected longitudinally and the colorless parenchymatous tissue was scraped out, liquified in a food-blender at 10, 000 g to remove the fibers. The resultant solution was lyophilized and stored at 4°C (Yagi *et al*., 1998).

Known amounts of the lyophilized powder were extracted with 95% ethanol at a temperature not exceeding the boiling point of the solvent (Lin *et al*., 1999). Nearly 85% of the solvent was recovered by distillation over the boiling water bath at atmospheric pressure and the remaining under reduced pressure in a rotavapor. The resultant residue was stored in a freezer at -80°C until use. Known amount of solvent free sample was suspended in water to obtain the desired concentration and subjected to antimicrobial studies.

Microorganisms

Both gram (+)ve and gram (-)ve bacteria and fungi (Table 1-3) were obtained from the culture maintenance division, Department of Medical Microbiology, University of Madras, Chennai, India.

Determination of Antibacterial Activity

Antibacterial screening test was performed by well-diffusion method using Nutrient Agar (NA) medium as described by Booth (1971). Hundred microliter of suspension containing 10^8 colony forming units mL^{-1} of bacteria spread on Nutrient Agar (NA) medium. Wells (4.0 mm) were punched out of the solid Nutrient Agar using pipette tips and different concentrations of the extracts and control antibiotics were placed into each well. Petri dishes were incubated at 37°C for 20 h and the average diameter of the inhibition zone surrounding the wells was determined visually.

Determination of Antifungal Activity

Antifungal activity was carried out by disc-diffusion method (Murray *et al*., 1995). Hundred microliter of suspension containing 10^4 spores mL^{-1} of fungi was spread on Potato Dextrose Agar

Table 1: Antimicrobial activity of *Aloe vera* gel extracts[a] against the gram (+)ve bacterial strains tested based on well diffusion method

	Aqueous extract		Ethanolic extract			
Microorganism	25 µg	50 µg	25 µg	50 µg	Antibiotics[b]	
Bacillus subtilis	-	+	++	++	Ofloxacin	Chloramphenicol
				++	++	++
Bacillus cereus	-	+	++	++	Ofloxacin	Chloramphenicol
				++	++	++
Bacillus megaterium	-	-	++	++	Chloramphenicol	Streptomycin
				++	++	++
Staphylococcus aureus	-	+	++	++	Sulbactam	Ciprofloxacin
				++	++	++
Streptococcus pyogenes	-	+	+	++	Ofloxacin	Ciprofloxacin
				++	++	++
Enterococcus faecalis	-	-	++	++	Gentamycin	Streptomycin
				++	++	++

[a] Antimicrobial activity :-, No inhibition; +, Zone of inhibition ≤8 mm in diameter; ++, Zone of inhibition >8 mm in diameter. [b] Antibiotics: Streptomycin (10 µg/well); Ofloxacin (10 µg/well); Sulbactam (30 µg/well); Ciprofloxacin (5 µg/well) Gentamycin (10 µg/well); Chloramphenicol (10 µg/well)

Table 2: Antimicrobial activity of *Aloe vera* gel extracts[a] against the gram (-)ve bacterial strains tested based on well diffusion method

	Aqueous extract		Ethanolic extract		Antibiotics[b]	
Microorganism	25 µg	50 µg	25 µg	50 µg	Streptomycin	Gentamycin
Escherichia coli	-	-	+	++	-	++
Salmonella typhii	-	-	+	++	++	-
Agrobacterium tumefaciens	-	+	++	++	-	-
Pseudomonas aeruginosa	-	-	+	++	++	-
Proteus mirabilis	-	+	+	++	-	-
Proteus vulgaris	-	-	-	+	-	-

[a] Antimicrobial activity :-, No inhibition; +, Zone of inhibition ≤ 8 mm in diameter; ++, Zone of inhibition > 8 mm in diameter [b]Antibiotics :Streptomycin (10 µg/well); Gentamycin (10 µg/well)

Table 3: Antimicrobial activity of *Aloe vera* gel extracts[a] against the fungal strains tested based on disc diffusion method

	Aqueous extract		Ethanolic extract			
Microorganism	25 µg	50 µg	25 µg	50 µg	Antibiotics[b]	
Aspergillus fumigatus	-	+	++	++	Tioconazole	Ketoconazole
				++	++	++
Microsporum canis	-	-	-	+	Fluconazole	Ketoconazole
				-	-	-
Aspergillus niger	-	+	++	++	Fluconazole	Ketoconazole
				++	++	++
Aspergillus flavus	-	-	+	+	Sulbactam	Netilmicin
				-	-	-
Fusarium oxysporum	-	-	++	++	Netilmicin	Ketoconazole
				-	++	++

[a]Antimicrobial activity: -, No inhibition; +, Zone of inhibition ≤8 mm in diameter; ++, Zone of inhibition >8 mm in diameter. [b] Antibiotics: Tioconazole (30 µg/disc); Ketoconazole (30 µg/disc); Fluconazole (30 µg/disc); Sulbactam (30 µg/disc); Netilmicin (30 µg/disc)

(PDA) medium. The plates were allowed to dry for 10-15 min. Sterile paper discs of 6 mm in diameter from whatman No.1 was prepared and sterilized by autoclaving. The discs were impregnated with the *Aloe vera* gel extracts from a stock of 1 mg mL^{-1}. Each disc contained either 25 or 50 µg of extract, respectively. Similarly discs impregnated with suitable concentrations of standard commercial antibiotics as described by Matsen (1979) were also prepared and used for susceptibility test. The discs were firmly pressed on to the agar surface of each seeded plate. Plates were incubated aerobically at 25°C for 72 h for fungi. Antifungal activities were measured and indicated by clear zones of inhibition against the test organism.

Antimicrobial activities of the gel extracts were expressed by – (no zone of inhibition), + (zone of inhibition = 8 mm in diameter) and ++ (zone of inhibition >8 mm in diameter). All the tests were performed in duplicate and repeated for confirmation of results.

Results and Discussion

The present study represents the most systematic study on the antimicrobial properties of *Aloe vera* leaf gel against multiple drug resistant common pathogenic bacteria and fungi and support the view that *Aloe vera* is a potent antimicrobial agent.

The effect of ethanolic extract of *Aloe vera* gel on *in vitro* antibacterial and antifungal activities against several pathogenic microbial isolates of clinical importance were presented in Table 1-3. Greater and remarkable antimicrobial activities were recorded with ethanolic extract even at low concentration (25 µg). The potency of the extracts was assessed by the presence or absence of inhibition zones and was presented in Fig. 1a-1f, respectively.

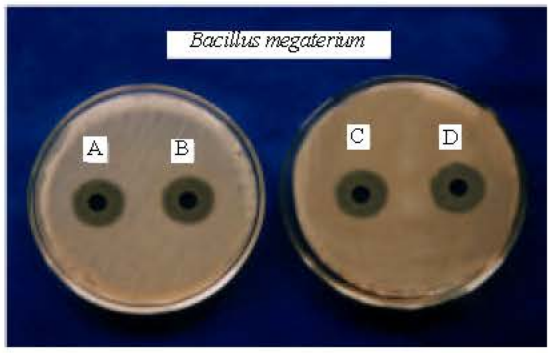

Fig. 1a: *Bacillus megaterium* showed high sensitivity to ethanolic extract of *Aloe vera* (A-25 g, C-50 g) and antibiotics Chlorampenicol (B-10 g/well) Strepotomycin (D-10 g/well)

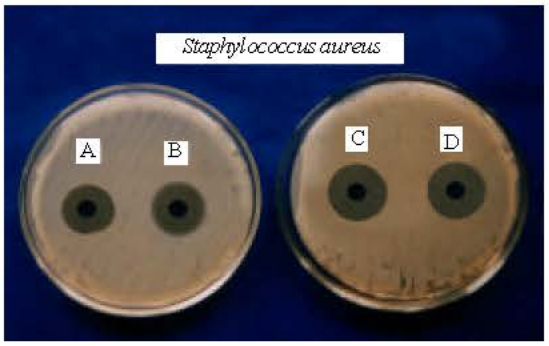

Fig. 1b: *Staphylococcus aureus* showed high sensitivity to ethanolic extract of *Aloe vera* (A-25 µg, C-50 µg) and antibiotics Sulbactum (B-30 g/well) and Ciprofloxacin (D-5 g/well)

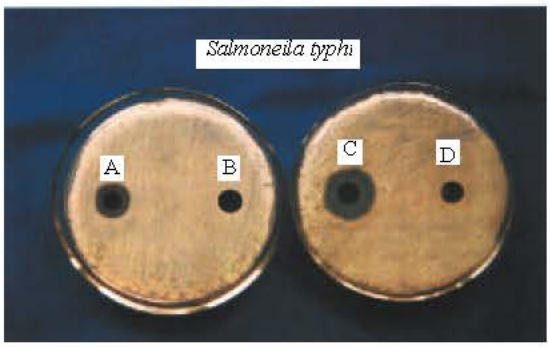

Fig. 1c: *Salmonella typhi* showed sensitivity to ethanolic extract of *Aloe vera* (A-25 µg, C-50 µg) and insensitivity to antibiotics Streptomycin (B-10 µg/well) and Gentamycin (D-10 µg/well)

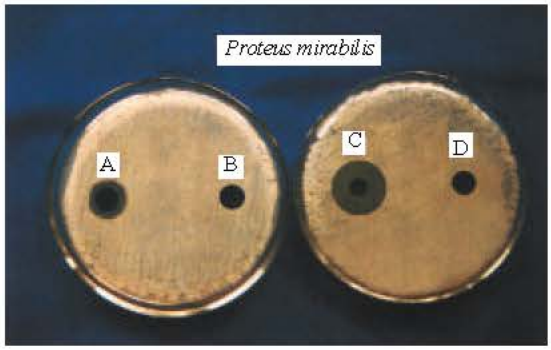

Fig. 1d: *Proteus mirabilis* showed sensitivity to ethanolic extract of *Aloe vera* (A-50 µg, B-50 µg) and in sensitivity to antibiotics Streptomycin and Gentamycin (B, D-10 µg/well)

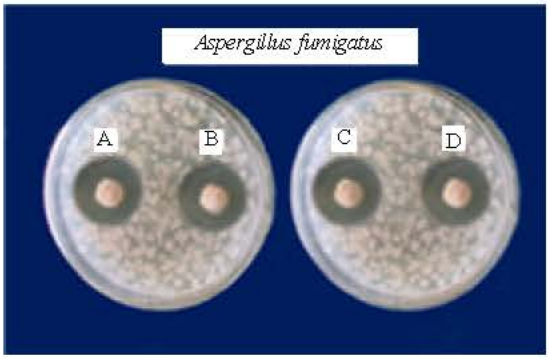

Fig. 1e: *Aspergillus fumigatus* showed high sensitivity to ethanolic extract of *Aloe vera* (A-25 µg and C-50 µg) and antibiotics Tioconazole (B- 30 µg/disc) and Ketoconazole (D- 30 g/disc)

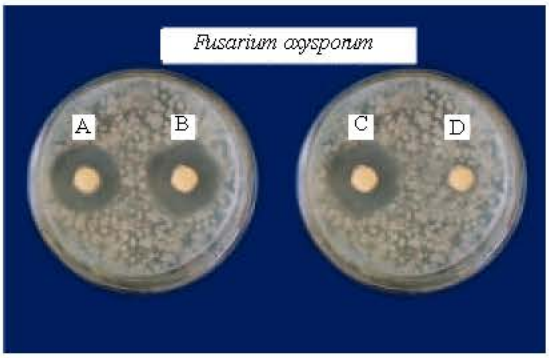

Fig. 1f: *Aspergillus fumigatus* showed high sensitivity to ethanolic extract of *Aloe vera* (A-25 µg and C-50 µg) and antibiotics Ketoconazole (B-30 µg/disc) and insensitivity to Netilmicin (D-30 µg/disc)

In recent years, multiple drug resistance in human pathogenic microorganisms have developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientists for searching new antimicrobial substances from various sources, like medicinal plants, which are the good sources of novel antimicrobial chemotherapeutic agents.

Lack of maximum inhibitory effect against, Gram-negative bacteria can be accounted for by the presence of their thick murein layer, which prevents the entry of growth inhibitors (Martin, 1995) or inherited genes on plasmids that can be easily transferred among bacterial strain.

The mechanism of action of the gel extract on the lysis of bacterial cells may be due to the pore formation in the cell wall and the leakage of cytoplasmic constituents by the active components such as alkaloids present in the gel extract as revealed by Shelton (1991). There are over 75 known ingredients in the *Aloe vera* leaf gel and they are all contained in about 1% of the plant, the rest (99%) being water, so they are obviously present only in small amounts. Their action is thought to arise from the synergistic effect of these substances i.e., they can be likened to work together as a team so that the total effect is greater than would be expected from the individual effect of each substance. Thus it was not surprising that *Aloe vera* leaf gel extract was highly prized just for this reason. Reports suggest that the beneficial effects of *Aloe vera* gel are due to its high molecular weight compounds such as polysaccharides (Shida *et al*., 1985), lectin-like proteins (Grindlay and Reynolds, 1986) and prostaglandins (Ali *et al*., 1991). Aloe's anti-inflammatory effects may be due to a bradykinin-degrading glycoprotein (Yagi *et al*., 1987) and mannose-6-phosphate may have a role in the wound healing process (Davis *et al*., 1994). Anthraquinones from various plant extracts have been studied for their possible anti-viral properties (Sydiskis *et al*., 1991).

Ethnomedicinally, the bacteria that are used in the present study for antimicrobial activity are known to be associated with the incidence of gastrointestinal tract, urinogenital tract and wound infections. Thus the present study adds credence to the ethnomedicinal uses of the plant for the treatment of gastrointestinal disorders.

The results of the present study also explains the use of this plant in folk medicine for the treatment of various diseases whose symptoms might involve microbial infections and underline the importance of the ethnobotanical approach for the selection of *Aloe vera* in the discovery of new bioactive compounds. From the above results, it may be concluded that *Aloe vera* gel extract possesses compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious diseases in humans. The Minimum Inhibitory Concentration (MIC) studies indicate that ethanolic extract of *Aloe vera* leaf gel was found to be active against most of the pathogenic bacteria and fungi even at very low doses.

The discovery of a potent herbal remedy that is safe will be a big advancement in fungal infection therapies. It is vital for systemic fungal infections that are usually common in immuno-compromised patients as toxicities induced by common antifungal drugs are often observed in these patients due to the high dosage and prolonged therapy. The presence of anthraquinones, saponins, tannins, lectins and alkaloids in the extract may be attributed to the antifungal actions of *Aloe vera*, since the antimicrobial actions of most of these phytochemical substances have been well documented (Deeni and Hussain, 1991; Shale *et al*., 1999).

Furthermore, besides the confirmation of the popular use, the obtained results demonstrate that this plant extract could represent a new source of potent anti-microbial, nontoxic, less expensive than the allopathic drugs. Further studies are in progress to isolate the active principle(s) which are responsible for the antibacterial and antifungal properties of *Aloe vera* leaf gel and to elucidate their mechanism of action.

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