Phytology

Phyto-chemical analysis and antimicrobial evaluation of aloe vera gel against some human and plant pathogens

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Abstract

Aloe Vent leaf gel is widely used as a traditional folk medicine for the treatment of different infectious diseases. Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides present in the gel of leaves. The study is aimed to investigate the phytoconstituents and antimicrobial activity of aqueous, ethanol and acetone extracts of the *Aloe vera* gel against some human and plant pathogens by the diffusion method. The extracts were screened for the presence of endotoxm contamination by Limukisamebocyte lysate test. The study showed that among three extract ethanol and acetone extracts recorded significant antimicrobial activity against all test pathogens. Antibacterial and antifungal activity of the acetone extract was found to be quite impressive as compared to ethanol and aqueous extracts. Ethanol extract showed high inhibition activity against *Bacillus Subtilis* (15.10 ± 0.01), *Micrococcus kristinae (5.22*±0.02) compared to acetone extract and low inhibition activity against *Candida albicans* (9.96 ± 0.2). *Microsporumcanis* (7.27 ± 0.02) surprisingly aqueous extract failed to show significant activity against all *Bacillus Subtilis* (7.96 ± 0.01). *Bacillus Cercus* (6.89±0.03), *Staphylococcus aureus* (7.89 ± 0.02), *Staphylococcus pyogenes* (8.0 ± 0.01), *Proteus mirabilis* (7.23±0.01), *Aspergillums fumigates* (6.93±0.13). *Aspergillums niger* (7.16 ± 0.2). *Aspergillums glaceans* (7.25 ± 0.02), *Tricophytommentagrophyte* (6.69±0.20), it showed very low inhibition activity indicating that the active principle responsible for antimicrobial activity is more soluble in organic solvents/The results suggested that *Aloe vera* gel extract with acetone can be used as antimicrobial agent against human and plant pathogens for medication, cosmetic and food purposes.

Keywords: *Aloe vera* gel, phytoconstituents, antimicrobial activity, human and plant pathogens

Introduction

Herbal medicines represent one of the most important fields of traditional medicine- all over the world. Promoting the proper use of herbal medicine and essential to study medicinal plants, which have folklore repution in a more intensified way, is required (Parekh and Chanda, 2007) [1]. Contrary to the synthetic drugs, antimicrobials of plants origin are not associated with side effects and have an enormous therapeutic potential to heal many infectious diseases for example, digitalis, ephedrine and vincristine were all originally discovered through research 01. plants. The potential for developing antimicrobials from higher plant appears regarding development of a Phyto medicine to act against microbes-Plant-based antimicrobials ha\e enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic anti microbials (Iwu *et al.*, 1999) [2].

Aloe vera is a member of Lilia cease family. *Aloe vera* (L.) Burm. Fil (Synonym Aloe berbadensis Miller) (TheIugu-Kalabandha, Tamil-Southakathalai-Hindi-Gikanvar) is a cactus like plant with green, dagger-shaped leaves that are fleshy, tapering, spiny, marinated and filled with a clear viscous gel (Yatters, 2002) [3]. The name was derived from the Arabic 'alloeh' meaning "bitter' because of bitter liquid found in the leaves. It is also known as lily of the desert' the plant of immortality and the medicine plant with qualities to serve as alternate medicine. *Aloe vera* is as old as civilization and throughout history it has been used as a popular folk medicine. It is present in the regions of India and is believed to be effective in treating stomach ailments, gastrointestinal problems, skin disease, constipation, radiation injury, inflammatory effect, healing wounds and burns, ulcer and diabetes. Currently the plant is widely used in skin care, cosmetics and as nutraceuticals (Klein and Penneys, 1988) [4], in this present study the gel *of Aloe vera* leaves were investigated for their phyto-chemical compounds and antimicrobial activity against some human and plant pathogens.

Materials and methods

Collection of plant material

Leaves *of Aloe vera* were collected from Mayor's house in Lodu Ndume Umuahia Abia State and identified by the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State.

Preparation *of Aloe vera* gel extracts

Mature, healthy and fresh leaves *of Aloe vera* having a length of approximately 2-3 feet were washed with hot water(40^0C), dissected longitudinally and the colorless parenchymatous tissue was scraped out, liquefied in a food blender to remove the fibers. The resultant solution was lyophilized and stored at 4^0C (Yagi *et al.*, 2014) [5]. Known amounts of the lyophilized powder were extracted with Distilled water, Ethanol (95%) and Acetone at a temperature not exceeding the boiling point of the solvent (Lim *et al.*, 1999) [6]. The solvent was recovered by distillation over the boiling water bath at atmospheric pressure and the remaining under reduced pressure in Rota vapor. The resultant residue was stored in a freezer at -80^0C until use. Known amount of solvent free sample was dissolved in DMSO to obtain the desired concentration and subjected to antimicrobial studies.

Endotoxin assay

Presence of bacterial endotoxin in all three extracts (Aqueous, Ethanol and Acetone) were measured by LAL assay using an E-TOXATE kit (Sigma, St. Louis, USA) as per manufacturer's instructions. Briefly, each extracts were incubated serially with LAL and chromogenic substrate, detection of endotoxin was measured by generation of ρ-nitroaniline at 405 nm and quantified against a standard curve of supplied bacterial endotoxin.

Human pathogenic microbial cultures

Bacterial cultures

Bacillus Subtilis, Bacillus Cereus, Bacillus Megaterium, Enterococcusfacalis, Staphylococcus aureus, Staphylococcus pyogenes, Staphylococeus epidermidis, Micrococcus krlstinae, Agrobacterium tumefaciens, Escherichia coli, Entrobacter aerogenes, Pseudomonas aeruginosa, Proteus tnirabilis, Proteus vulgaris, Salmonella typhi, Shigellsonnei were obtained from the Department of Microbiology, Nizam Medical college. Hyderabad. India. All the test strains \\ere maintained on nutrient agar slope (Hi-Media Laboratories Pvt. Limited. Mumbai, India) at room temperature and were sub-cultured into newly prepared nutrient agar slants, every two-weeks.

Fungal culture

Aspergilhisfumigatus, Aspergillus Niger, Aspergillus flavus, Aspergillus glaceans, Candida tropicalis, Candida albicans, Fusariumoxy sporum, Micro sporumcanis, Tricophytommentagrophyte were obtained from the Department of Microbiology, Nizam Medical College, Hyderabad, India. All the fungal strains were maintained on malt extract-agar, while *Candida tropicalis, Candida albicans* are maintained on Sabouraud-dextrose-agar (Hi-Media Laboratories Pvt. Limited, Mumbai, India) at room temperature and were sub - cultured, every two weeks.

Plant pathogenic microbial cultures

Pseudomonas syringe, Xanthomonas camestris, Colletotrichum coccodes, Fusariiiin.oxyspon.im, Rhizoctoniasolani these test strains were isolated from tomato, potato crops by the hyphae point and monoscopic techniques. The isolate strains were maintained on potato-dextrose-agar (Hi-Media Laboratories *Pvt.* Limited, Mumbai, India) at room temperature. All the microbial cultures were served as test pathogens for the assay.

Disc preparation

The 6mm (diameter) discs were prepared from what man No. l filter paper and the discs were sterilized by autoclave at 12I°C. After sterilization the moistened discs were kept in hot air oven at 50 C (Satish *et al.*, 1999) [7]. Then discs were impregnated with suitable concentration of the *Aloe vera* gel extracts from stock of 1 mg/ml. Each disc contained 50mg of extract.

Determination of antibacterial activity

Antibacterial activity was carried out by disk-diffusion method using nutrient Agar medium (Booth, 1979) [8]. Hundred micro liter of suspension containing 10^8 colony forming units ml of bacteria spread over the nutrient agar medium plates by using separate sterile cotton buds. After the microbial lawn preparation three different extract of plant discs (aqueous, ethanol and acetone extract) were firmly pressed on to the agar surface of each seeded plate. Petri dishes were incubated at 37^0C for 24h and the average diameter of the inhibition zone surrounding the wells was determined visually.

Determination of antifungal activity

Antifungal activity was carried out by disk-diffusion method (Roberts *et al.*, 1981) [9] hundred micro liter of suspension containing 10^4 spores mL^{-1} of fungi was spread on Potato Dextrose agar (PDA) medium. The plates were allowed to dry for 10-15 mm. After the microbial lawn preparation of different extract of plant discs were placed on the organism inoculated plates. The plates were incubated aerobically at 25^0C for 72 hours for fungi. Anti-fungal activities were measured and indicated by clear zones of inhibition against the test organism. The diameter of the minimum zone of inhibition was measured in mm. For each test, three replicates were performed.

Phytochemical analysis

Phytochemical analysis of Ethanol, Acetone and Water extracts for presence/absence of metabolites such as carbohydrates, alkaloids, glycosides, Flavonoids, tannins, steroids, saponins, triterpenoids, and Phenolic anthraquinones was carried out (Roberts *et al.*, 1981) [10].

Statistical Analysis: Data were expressed as mean ± standard deviation. The data obtained were subjected to ANOVA test to determine whether there was significant difference between extract used and also between the lengths of incubation.

Results

Antimicrobial activity of different extracts of *Aloe vera* gel (ethanol, acetone and aqueous) at 50uL concentration against some important human and plant pathogenic micro organisms were presented in table 1-4 LAL assay showed the amount of endotoxin to be 0.01 IU/ml in all the three extracts indicating a negative result. This confirms very negligible amount of endotoxins in the extracts which were screened against human and plant pathogens.

Antibacterial activity (Table 1 and 2)

Antibacterial activity of *Aloe vera* gel was analyzed against eight gram +ve, eight gram-ve pathogenic strains. Among three extract ethanol, acetone extracts recorded significant antibacterial activity against all test pathogens, the maximum antimicrobial activities were observed in acetone extract other than ethanol and aqueous extract. Antibacterial activity of the acetone extract of *Aloe vera* gel was found to be quite impressive as compared to ethanol and aqueous extracts. However, high inhibition activity against *Staphylococcusaureus* (15.98 ± 0.02), *Staphylococcuspyogencs* (16.40 ± 0.02), *Staphylococcus epidermidis* (14.0 ± 0.03), *Escherichiacoli* (15.03 ± 0.02), *Pseudomonasa eruginosa* (16.04 ± 0.03), *Proteusmirabilis* (14.12 ± 0.02), *Proteusvulgaris* (15.09 ± 0.04) was observed, moderate inhibition activity against *Bacillus Subtilis* (10.80 + 0.01), *BacillusCereus* (10.25 ± 0.02), *Bacillus Elaterium* (10.90± 0.03), *Enterococcus acalis* (10.10 ± 0.01), *Agrobacterium tumefaciens* (11.39 ± 0.05), *Salmonella typhi* (10.77 ±0.03) and low inhibition activity against *Shigellsonnei* (7.25 ± 0.02), *Entrobactera erogenes* (7 34 ± 0 02). In case of ethanol extract it showed inhibitory activity against all test pathogens but it showing high inhibition activity against *Bacillus Subtilis* (15.10 ± 0.01), *Micrococcus kristinae* (15.22 ±0.02) compared to acetone extract, surprisingly aqueous extract could not showing any inhibitory effect against all pathogens except *Bacillus Subtilis* (7.96 ± 0.01), Bacillus Cercus (6.S9 ± 0.03), *Staphylococcus aureus* (7.89±0.02), *Staphylococcus pyogenes* (8.0 ± 0.01), *Escherichia coli* (6.21 ± 0.05), *Proteus mirabilis*, (7.23 ± 0.01) it showing very low inhibition activity.

Results

Table 1: Antibacterial activity of *Aloe vera* gel extracts against the gram (+) ve bacterial strains tested based on disc diffusion method

Micro organism	Aqueous extract(50μg)	Ethanol extract (50 μg)	Acetone extract(50μg)
Bacillus subtilis	9. 96 ±0.02	17.10 ±0.00	12.08 ±0.02
Bacillus Cereus	7. 89 ±0.05	10.26 ± 0.02	11.18 ± 0.04
Bacilnsmegaterium	-	12.32 ± 0.03	12.07 ± 0.02
Enterococcusfacalis	-	10.34 ± 0.04	11.10±0.04
Staphylococcnsaureus	6.89 ±0.05	13.07 ± 0.05	18.93 ±0-04
Siuphylococcuspyogenes	7.0±0.03	14.00 ± 0-03	17.42 ±0.05
Staphylococcitsepidermidis	-	9.98 ± 0.04	15.03± 0.05
Micrococcus kristinae	-	17.22 ±0.05	10. 22 ±0.03

Antibacterial activity; Zone of Inhibition (nun in diameter) (Mean +. SI)) (n=3). - - Negative antibacterial activity (no zone of inhibition)

Table 2: Antibacterial activity of *Aloe vera* gel extracts against the gram (-} ve bacterial strains tested based on disc diffusion method

Micro organism	Aqueous extract (50μg)	Ethanol extract (50μg)	Acetone extract (50μg)
Agrobacterium tumefaciens	-	13.43 ± 0.02	17.39 ±0.05
Escherichia coli	8.11 ±0.02	10.34 ±0.02	16.03 ±0.02
Entrobacteraerogenes	-	9.54±0.02	9.34 ± 0.02
Pseudomonas aeruginosa	-	13.18 ±0.03	18.04 ±0.03
Proteus mirabilis	8.13 ± 0.02	13.34 ± 0.02	16.11±0.03
Proteus vulgaris	-	10.87 ±0.03	17. 13±003
Salmonella typhi	_	11.53 ± 0.03	14.12±0.02
Shigell sonnet	-	8.32 ±0.02	11.20±0.03

Antibacterial activity: Zone of Inhibition (mm in diameter) (Mean +SD) (n=3)

Anti fungal activity (table 3), anti fungal activity of *Aloe vera* gel was analyzed against nine gungal pathogenic strains. Amongst three extract ethanol, acetone extracts recorded significant antifungal activity against all test pathogens, the maximum antifungal activities were observed in acetone extract other than ethanol and aqueous extract. Antifungal activity of the acetone extract of *Aloe vera* gel was found to be quite impressive as compared to ethanol and aqueous extracts. However, high inhibition activity against *Aspergillus* fumigates (14.63 ± 0.05), Aspergillus flavus (15.27 ± 0.02) *was observed, moderate inhibition activity against* Aspergi/tus Niger *(10.20 ± 0.03),* Aspergillusglaceaus (11.06 ± 0.11), Candida tropicalis (11.94 ± 0.04), Candida albicans *(10.20 + 0.03),* Fiisariumoxysporum *(10.83 ± 0.02),* Microsporumcanis (11.20 ± 0.04). *In case of ethanol extract it showing inhibition activity against all test pathogens but it showing low inhibition activity against* Aspergilhtsflavus (11.57 ± 0.02), Micro sporum cams (7.27 ± 0.02). *Surprisingly aqueous extract could not showing any inhibitory effect against all pathogens except in* Aspergillusfumigatus (7.25 ±0.02), Aspergillus Niger (7.16

± 0.2), Aspergillusghiceaus (6.93 ± 0.13), Tricophytommentagrophyte (6.69 ± 0.20) it showing very low inhibition activity.

Table 3: Anti-fungal activity of *Aloe vera* gel extracts against the fungal strains tested based on disc diffusion method

Micro organism	Aqueous extract (50µg)	Ethanol extract(50µg)	Acetone extract(50µg)
Aspergillus fumigates	7.25 ± 0.02	11. 12 ±0.03	14.63+0.05
Aspergillusniger	7. 16 ±0-02	10.10 ±0.21	10,20 + 0.03
Aspergillusflavus	~	11. 57 ±0.02	15.27 + 0.02
Aspergillusglaceans	6.93 ±0.13	10.63 + 0.04	11.06+0.11
Candida tropicalis	-	11.1 ±0.04	11 94 +0.04
Candida alblcans	-	9.96 ±0.21	10.20 + 0.03
Fusariumoxysponim	-	10.49 + 0.05	10.83 ±0.02
Micro sponimcanis	-	7. 27 ±0.02	11.20 ±0.04
Tricophytommentagrophyte	6.69+0.20	10.21 ±0.28	11.61 ±0.01

Anti-fungal activity: Zone of Inhibition (mm in diameter) (Mean ± SD) (n-3), - = Negative anti-fungal activity (no zone of inhibition)

The result of *Aloe vera* gel extracts against the plant pathogens showed (Table 5) that among three extract ethanol, acetone extracts showed an inhibitory effect against all test pathogens. Surprisingly, aqueous extract could not show any inhibitory effect against all pathogens.

Table 4: Antimicrobial activity of *Aloe vera* gel extracts against the plant pathogenic strains tested based 01 disc diffusion method

Micro organism	Aqueous extract(50µg)	Ethanol extract(50 µg)	Acetone extract(50 µg)
Pseudomonas syringe	-	12.13 ±0.02	15.15 ± 0.02
Xanthomonascamestris	-	9.23 ± 0.03	9.45 ±0.01
Colletotrichumcoccodes	-	10. 35±0.02	13. 24 ±0.01
Fusarhim oxy sporum	-	11.13±0.02	14.13±0.02
Rhizoctoniasolani	-	10.21±0.02	12.33±0.01

Antimicrobial activity: Zone of Inhibition (mm in diameter) (Mean ± SD) (n=3), Negative antibacterial activity (no zone of inhibition)

Phytochemical evaluation

Phytochemical analysis of all the extracts revealed that Carbohydrates, Glycosides, Flavonoids Phenolic atathraquinon.es, Tannins and Saponins are generally present in all the extract. Triterpenoids were found in Acetone and. Ethanolic extracts. Other metabolites such as Alkaloids, Steroids were absent in all the extracts (Table 5).

Table 5: Preliminary Phytochemical analysis of *Aloe vera* gel extracted with different solvents

Phytoconstituents	Test	WE	AE	EE
Carbohydrates	Molish Test	+	+	+
	Dragnedroft	-	-	-
Alkaloids	Mayer	-	-	-
	Wanger	-	-	_
Glycosides	Glycoside test	+	+	+
Flavonoids	Flavonoid test (0.5% KOH alch)	+	+	+
Tannins	Tannins test (l% Pb $(OAC)_2$)	+	+	+
Steroids	Steroid test	-	-	-
Saponins	Saponine test (5%Hgcb	+	+	+
Triterpenoids	Triterpenoids test	-	+	+
Phenolic anthraquinones	Phenoletest (l% $FeCl_3$)	+	+	+

+ Present. - Absent, WE-Water extract, AE-Acetone extract. EE-Ethanol extract

Discussion

Antibiotics provide the main basis for the therapy of microbial infections. However, the high genetic variability of bacterial enables them to rapidly evade the action of antibiotics by developing antibiotic resistance In recent years development of multidrug resistance in the pathogenic bacteria and parasites has created major clinical problems in the treatment of infectious diseases (Davis, 1994) [11]. This and other problems such as toxicity of certain antimicrobial drugs on the host tissue (Onuoha *et al.,* 2023; Onuoha and Alaebo, 2022) [12, 13] triggered interest in search of new antimicrobial substances drugs of plant origin. Considering the rich diversity of plants, it is expected that screening and scientific evaluation of plant extracts for their anti-microbial activity may provide new anti-microbial substances; hence the present investigation clearly reveals the antimicrobial nature of this plant and suggests that this plant could be exploited in the management of diseases caused by these microbes in both plant and human systems. It is interesting to note that antimicrobial activity was highly pronounced in solvent extracts compared to aqueous extract. It is also important to note that susceptibility of the pathogens was varied to solvent extract and aqueous extract. This indicates the presence of more than one active principle in *Aloe vera* gel. Plants are rich reservoir of antimicrobials (Cowan, 1999) [14] it is observed that a single plant is known to contain several active principles of biological significance (Ming, 2005) [15]. The present finding is hence highly encouraging in recognizing a plant of interesting antimicrobial activity.

Numerous aloe species around the world are used for conditions ranging from dermatitis to cancer (Kemper and Chiou, 1999) [16]. Furthermore, the fresh gel, juice or formulated products have been used for medical, food and

cosmetic purposes, as well as for general health (Park and Joe, 2006) [17]. In the last decade, *Aloe vera* has been used extensively in health drinks, topical creams, toiletries and cosmetics (Anos, 2008) [18] and there are many reported claims of its beneficial properties, encompassing a broad range of conditions (Reynolds and Dweck, 1999) [19]. There is a wide range of research from all over the world based upon different species of Aloe for antimicrobial activity. In the study, *Aloe vera* gel had inhibitor, effect against some diseases caused pathogens in human and plants. Among three extract (ethanol, acetone and. aqueous extracts) ethanol, acetone extracts recorded significant antimicrobial activity against all test pathogens, the maximum antimicrobial activities were observed in acetone extract other than ethanol and aqueous extract-Antibacterial and antifungal activity of the acetone extract of *Aloe vera* gel was found to be quite impressive as compared to ethanol and aqueous extracts. In case of ethanol extract u showing inhibition against all test pathogens but it showed high inhibition activity against *Bacillus Sublilis, Micrococcus krisrinue* compared to acetone extract and low inhibition activity against *Aspergi Husflavus, Microsponim cams* surprisingly aqueous extract failed to show any inhibitory effect against all pathogens except in *Bacillus Subtilis, Radius Cereus, Bacillus Megaterium, Staphylococcitsaitreus, Staphylococcus pyogenes, Proteus mirabiHs, Aspergi Husfumigates, Aspergillus njser, Aspergillus glaceaus, Tricophytommentagrophvte.* Regarding the possibility of presence of endotoxins' in the extracts many literatures in both *in vitro* and *in vivo* studies revealed below detectable level of endotoxin level in *Aloe vera* gel (Avijit, 2008) [20]. As the extracts were significantly active against plan pathogens, it may be employed as a promising pesticide against plant pathogens which were under taken in our study. Further studies are to be under taken to establish the pesticidal activity of *Aloe vera* gel extracts. 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 constituent by the active components such as alkaloids present in the gel extract as revealed by Shelton (Shelton, 1991) [21]. 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) [22], lectin-like proteins (Gandley and Reynolds, 1986) [23] and prostaglandins (Ali *et al.,* 1991) [24]. Aloe's anti-inflammatory effects may be due to a bardykinin, degrading glycoprotem (Yagi *et al.,* 1985) [25] and mannose-6-phosphate may have a role in wound healing process (Davis *et al.,* 1994) [26]. Anthraquinones form various plant extracts have been studied for their possible anti-viral properties (Sydiskis *et al.,* 1991) [27]. The discovery of a potent herbal remedy that is safe will be a big advancement in fungal infection therapies. The presence of anthraquinones, saponins, tannins, lectins and alkaloids in the extract may be attributed to the antifungal actions of *Aloe vera*. 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 ethno botanical 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 extracts possesses compounds with antimicrobial properties which can be used as antimicrobial agents in new drugs for therapy of infectious disease in humans and plants. Further studies are in progress to isolation and estimation of active components responsible for the antimicrobial properties of *Aloe vera* leaf gel and to elucidate their molecular mechanism of action.

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