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Antioxidant and Antibacterial Potentials of *Aloe vera* Juice Extract against Wound Isolates

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Wound is a injury made on the skin due to torn, cut, trauma and contusion. Microorganisms are responsible for the severity in hospitalized wound. To evaluate the importance of *Aloe vera* on the wound pathogens this work was undertaken. The healing properties of the succulent plant *Aloe vera* have been known for thousands of years. This study was aimed at to evaluate the antibacterial and antioxidant activity of *Aloe vera* extract. Wound (pus) samples were collected from Government hospital Srirangam. Samples were processed and the microorganisms were isolated and identified using macroscopic, microscopic and biochemical analysis. *Escherichia coli, Staphylococcus aureus*, *Streptococcus pyogens* and *Pseudomonas aeruginosa* were isolated and identified as a predominant flora of wound infection. *Aloe vera* plant was chosen for screening its antibacterial activity. Antibacterial activity of *Aloe vera* extract was studied by disc diffusion method, which showed best activity against all clinical isolates at 200µg/ml concentration. Phytochemical analysis of the plant extract revealed the presence of flavonoids, tannins, phenolic compounds and steroids.

Key words: *Aloe vera,* wound, Pus, Antimicrobial activity, Antioxidants, Phytochemistry.

Aloe vera also known as the Barbadensis Aloe, a member of the family Liliaceae, is a stemless succulant plant. In Sanskrit it is called as "Ghrita Kumari". Traditionally *Aloe vera* is used topically to heal wounds and for various skin conditions. Historically this has been used for a variety of medicinal purposes[1].

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Aloe vera has been promoted for large variety of conditions and has come to play a prominent role as a contemporary folk remedy[2]. The fresh leaves of *Aloe vera* are also used as a laxative.

Microorganisms plays a major role in creating more severe and pyogenic wound among hospitalized and non hospitalized patients[3].

Antibiotic containing oinments and oral antibiotics were used for the treatment. Presently used antibiotic agents are failing to bring an end to many bacterial infections due to super resistant strains and side effects. People of developing and developed countries are at present turning towards Traditional System of Medicine. According to world health organization (WHO), 80% of the world population relies on plant drug[4] for their primary health care.

Aloe gel has proven wound healing[5-6], antitumour[7], antiviral[8] and antimicrobial[9] potentials. Besides aloe gel also acts as antioxidants. *Aloe* extracts may facilitate the creation of a flexible, fine scar with high tensile strength at the wound site[10].

Phytochemical analysis of *Aloe vera* extract showed the presence of highly effective bioactive compounds. These compounds are also reported to possess antibacterial, antifungal, anti inflammatory, anticancer and antidiabetic activity.

This study was undertaken to establish antibacterial and antioxidant potentials of *Aloe vera* juice extract against wound pathogens along with phytochemical constituents.

MATERIAL AND METHODS

Sample Collection for wound pathogen isolation

The pus samples from pyogenic wound cases were collected from out and inpatients of Government hospital, Srirangam in sterile wide mouthed containers and transported to the laboratory with in an hour for further processing and testing.

Selective Isolation

Pus samples were streaked on Mac Conkey agar, neomycin blood agar and Blood agar for the selective isolation of pathogens[10].

Identification

Isolated bacteria were identified based on colony morphology on Nutrient agar and Selective medium. Microroscopy was done to look for the shape, gram's nature and motility. Further identification was done by performing various biochemical tests[11]

Bacterial strain used

Staphylococcus aureus, Streptococcus pyogens, Pseudomonas auroginosa, Enterobacter sp., and *Eschericia coli.*

Antibiotic sensitivity assay

Twenty antibiotics were selected based on their potential use in patients. Susceptibility to antimicrobial agents was determined by the disc diffusion method according to the NCCLS guidelines[12]. Petriplates containing 20 ml of Mueller Hinton agar were seeded with 4 hours old fresh culture of clinical isolates and referral strains. Discs were purchased from Hi Media Laboratories PVT LTD, Mumbai, Zones of inhibition were measured after 18 to 24 hours of incubation at 37°C. Susceptibility breakpoints were determined according to NCCLS guidelines.

Processing of *Aloe vera*

The *Aloe vera* is processed in a special way to avoid the loss of essential vitamins, minerals and other constituents. The green outer portion of the leaf which contains aloin is removed, leaving the gel that remains in the leaf. The raw juice is prepared with the help of a highly sophisticated Aloe juice extraction where all operations are untouched by hand. Suitable biopreservatives and stabilizers are added and filtered using micro filters. The juice is hygienic and totally free from toxic pathogens. Juice is dehydrated to obtain extract and subjected to various studies

Antibacterial sensitivity assay of extracts

Preparation of disc

Known Quantity of *Aloe vera* dried juice extract was dissolved in DMSO in a ratio of 1:1. It was then filtered by making use of syringe filter of pore size 0.42µm. Sterile discs of 6mm diameter were loaded with various concentrations of extracts and were dried. Dried discs were stored in sterile containers till use. Solvent loaded discs were also prepared and were used as Negative control Streptomycin loaded discs were used as positive control.

Antibacterial assay

Petriplates containing 20ml of nutrient agar medium were seeded with a 24 hours old culture of the bacterial strain. 100µg, 200µg, 400µg and 800µg concentration of plant samples were impregnated into the sterile 6mm diameter discs. Discs were dried and dispensed on the solidified nutrient agar medium previously inoculated with test microorganisms. DMSO and tetracycline discs were used as positive and negative control. Incubation was made at 37^0C for 24 hours. The assessment of antibacterial activity was based on the measurement of diameter of the Inhibition Zone formed around the discs[12].

Determination of Minimum Inhibitory Concentration (MIC)

Agar dilution method was used to find out the minimum inhibitory concentration. MIC was recorded based on the growth of the test organism[12].

Antioxidant activity

Reducing power assay

The reducing power of *Aloe vera* juice extract was determined by reducing powder assay[13]. Various concentrations of the extracts (20 to 100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (2 to 10 µg/ml) was used as standard. Increased absorbance of the reaction mixture indicates increase in reducing power.

% increase in Reducing Power
= {A Test/ A Blank-1}X100

A test is absorbance of test solution;
A blank is absorbance of blank.

Phytochemical screening

Qualitative and quantitative analysis of secondary metabolites were done by making use of standard methods[14].

Statistical analysis

Statistical analysis was done by using Origin 6.0 software. Antimicrobial activity and antioxidant activities were assessed by calculating mean ± SD.

RESULTS AND DISCUSSION

Wound may be produced by physical, chemical, thermal or immunological lysis of the tissue[16]. In the present study, postoperative wound pus samples were collected (n=40) from (Fig. 1) cases admitted in Government hospital srirangam and 40 samples were collected from non hospitalized patients. Standard methods were adopted to isolate pathogens and identified. Among various isolates *Staphylococcus aureus* showed higher incidence (47%) followed by *Pseudomonas aeruginosa* (22%), *Enterobacter sp.,* (18%) and *Escherichia coli* (13%), where as Enterobacter and Escherichia coli were not isolated from non hospitalized patients. Staphylococcus aureus showed higher incidence in both the group of patients. This incidence may vary depending on immunity, climate and hygienic conditions (Fig. 2). *Escherichia coli* and *Enterobacter sp.* isolated from hospitalized cases could indicate that, they may be Nosocomial pathogens.

Pathogenic microorganisms build resistance against the common antibiotics. Sensitivity pattern of the organisms revealed that all the test organisms were resistant to more than 11 antibiotics tested (Table 1). A total of 20 different commercial antibiotics available in the market representing broad and narrow spectrum based on their activity and synthetic and semi synthetic nature of production were chosen for antibiotic sensitive assessment. The concentration of chosen antibiotics ranges from 5µg to 200µg. This concentration was based on commercial availability of the disc in the market. It was interesting to note that *Escherchia coli* was resistant to 13/20 antibiotics tested (65%), *Staphylococcus aureus.* was resistant to 12/20 antibiotics tested (60%), Streptococcus pyogenes to 14/20 (70%) and Enterobacter sp. to 11/20 antibiotics tried (55%). Only oxytetracycline was effective against all the pathogens tested. Multidrug resistance pattern of various isolates in hospital was increased by 50% during the 2001-2003 but it was only 7% during the year 2000[17].

Antibacterial sensitivity pattern of *Aloe vera* extract against pathogens like *E. coli, Pseudomonas aeruginosa, Streptococcus pyogens, Enterobacter sp* and *Staphylococcus aureus* revealed that Aloe extract inhibited all the pathogens effectively and the zone of inhibition ranged from 10.8±1.3mm to 15.6±0.8mm. Best activity was noted against *Streptococcus pyogenes* (15.6±0.8mm) at 800mg/ml concentrations (Table 2). Ferro *et al.,*[18] also reported similar kind of results using pathogens like *Streptococcus pyogenes, Shigella flexneri*, *K. pneumoniae* and *Aeromonas s*p.

MIC of *Aloe vera* extract against different pathogens ranging from 085±13 to 360±22 (Table 3). Enterobacter is best inhibited by Aloe extract followed by Staphylococcus aureus at 150±17. Present report also was in line with the report of Ferro *et al.*,[18].

Preliminary phytochemical reports revealed the presence of Flavonoids, steroids,

Table 1. Antibiotic sensitivity pattern of clinical isolates

S. No	Antibioticstested	Quantity used	Wound pathogens/Zone of inhibition in mm				
			S. aureus	*S. pyogenes*	*P. aeruginosa*	*E. coli*	*Enterobacter*
1	Amikacin	030µg	16	R	R	R	R
2	Azithromycin	015µg	15	R	15	15	R
3	Carbenicillin	100µg	20	22	R	R	R
4	Cefdinir	005µg	18	23	R	R	R
5	Cefixime	005µg	20	17	R	R	R
6	Chloramphenicol	030µg	23	23	23	R	19
7	Ciprofloxacin	005µg	R	R	29	R	28
8	Doxycycline	030µg	R	R	22	20	18
9	Levoflaxacin	005µg	R	R	R	R	R
10	Methicilline	005µg	R	R	12	10	11
11	Nalidixicacid	030µg	R	R	28	20	23
12	Novobiocin	030g	R	R	R	R	R
13	Ofloxacin	005µg	R	R	R	R	R
14	Sparfloxacin	005µg	R	R	R	R	R
15	Ticarcillin	075µg	R	R	R	R	R
16	Vancomycin	030µg	R	R	R	R	R
17	Clarithromycin	015µg	R	R	15	15	18
18	Gentamycin	010µg	13	13	13	R	25
19	Spectinomycin	100µg	R	R	R	19	20
20	Oxytetracycline	030µg	22	15	26	23	22

R- Resistant

Table 2. Antibacterial activity of *Aloe vera* extract dried extract against pathogens isolated from wound(pus) sample

S. No	Test organism	Concentration of extracts/zone of inhibition in mm (mean±SD)					
		Positive control	Negative control	100µg/ ml	200µg/ ml	400µg/ ml	800µg/ ml
1.	*Enterobacter sp,*	24	-	10.8±1.3	11.4±1.6	12.6±0.8	14.2±1.1
2.	*Escherichia coli*	28	-	Nil	Nil	12.6±1.6	14.2±1.3
3.	*Staphylococcus aureus*	30	-	Nil	12.4±1.1	13.0±1.5	16±2.0
4.	*Pseudomonas aeruginosa*	28	-	Nil	Nil	11.2±1.4	12.4±0.9
5.	*Streptococcus pyogens*	22	-	Nil	11±1.5	13.6±1.5	15.6±0.8

Positive control-Oxytetracycline; Negative control – DMSO; SD- Standard Deviation

quinones, Saponins, tannins. Terpenoids and phenolic compounds (Table 4). Quinone compound present in *Aloe vera* may be responsible for its antibacterial activity[16].

Quantitative phytochemical report showed that aloe extract possess flavanoids (1.71mg/kg) at higher amount followed by Tannins (0.22mg/kg), Quinones (0.22 mg/kg), Phenolic compounds (0.12 mg/kg), Steroids (0.07 mg/kg) and Phytosterol (0.02 mg/kg) (Table 5). Davis *et al.,*[16] also reported that Aloe *vera* contain tannins, Flavonoids, phenolic compounds, Quinones, saponins in considerable quantities.

The reductive capabilities of the *Aloe vera* extract were compared with ascorbic acid for the reduction of the Fe^{3+} - Fe^{2+} transformation. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the antioxidant activity of antioxidants have been attributed to

Table 3. Minimum Inhibitory concentration of *Aloe vera* extracts

S. No	Test Organism	MIC in µg/ml (mean±SD)
1	*Enterobacter sp,*	085±13
2	*Escherichia coli*	320±20
3	*Staphylococcus aureus*	150±17
4	*Pseudomonas aeruginosa*	360±22
5	Streptococcus pyogens	265±13

Table 4. Phytochemical Screening of *Aloe vera* Extracts

S. No	Test	Result
1	Alkaloids	-
2	Flavanoids	+
3	Steroids	+
4	Glycosides	+
5	Terpanoids	+
6	Tannins	+
7	Quinine	+
8	Coumarins	+
9	Starch	-
10	Saponins	+
11	Phenols	+

Table 5. Quantitative Analysis of Secondary Metabolites in *Aloe vera*

S. No	Phytochemicals	Quantity (mg/kg)
1	Tannins	0.22
2	Phenolic compounds	0.12
3	Steroids	0.07
4	Flavanoids	1.71
5	Quinines	0.22
6.	Phytosterol	0.02

Table 6. Antioxidant activity of *Aloe vera* extracts

S. No	Sample	Concentration (mg)	Reducing power (%mean ±SD)
1	*Aloe vera* extract	20	20.0±1.2
2		40	27.5±1.3
3		60	34.7±0.7
4		80	37.8±1.5
5		100	45.4±1.6
1	Ascorbic acid	2	8.7±1.2
2		4	11.6±1.4
3		6	25.5±1.6
4		8	37.6±0.9
5		10	45.3±1.8

SD- Standard Deviation

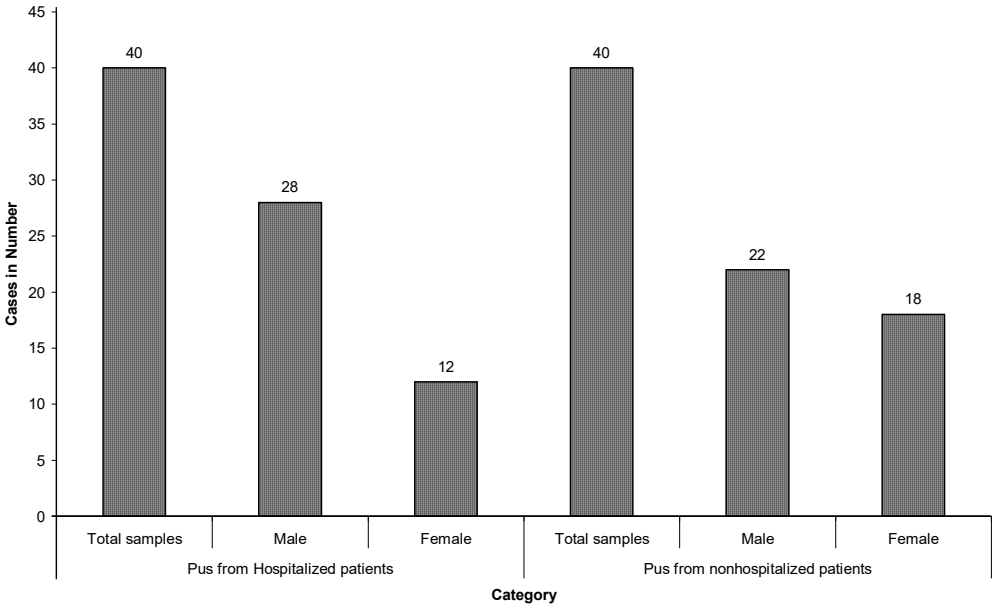

Fig. 1. Categories of Pus sample collection

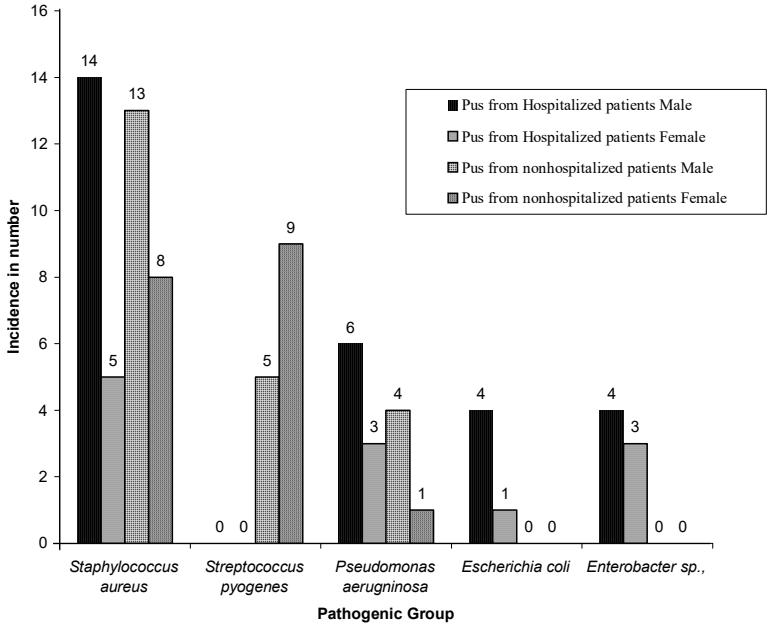

Fig. 2. Incidence of Microbial agents in wound patients

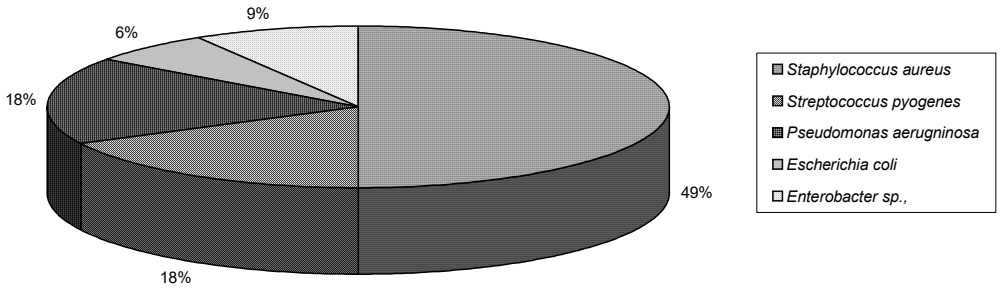

Fig. 3. Incidence of different pathogens among would patients

various mechanisms, among which are prevention of chain initiation of protein synthesis, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging antioxidant activity. The reducing power of aloe extract was increased with increasing amount of sample. The antioxidant activity has been reported to be concomitant with the development of reducing power. All the concentration of aloe extract showed significant activities when compared to standard ascorbic acid (Table 6).

REFERENCES

1. Akinyehe, S., Mat, Y. The properties of *Aloe vera . J. ethanopharmacol.,* 2007; **45**: 27-9.
2. Choi, S., Son, B., Park, Y. The wound healing effect of a glycoprotein fraction isolated from *Aloe vera*. B. *J. dermatol.,* 2002; **145**(4): 535-45.
3. Khurram, S.M., Shahzed, A.J., Shaista, N.K., Salman, S.E. Comparative antimicrobial activity of *Aloe vera* gel on microorganisms. *J. Med*. 2009; **7**: 416-23.
4. Jeywardiane, G., Kumara, O., Akuwihare, A. Complete colonic duplication in an infant. *J. med*., 2001; **46**: 69-70.

5. Davis, R., Parker, W., Murdoch, D. *Aloe vera* as a biological vehicle for hydrocortisone acetate. 1989; **81**: 1-9.
6. Heggers, J.P. Beneficial effect of *Aloe vera* on wound healing. *J. Alt. and comple. Med.,* 1996; **2**: 271-77.
7. Kim, A., Perece, E. Aloe emodin is a type of anticancer agent with selecting the activity against Neuroectodermal tumours. *Cancer Res.* 2000; **164**: 176-78.
8. Mcdaniels, H., Carpenter, R., Kemp, R. Extended survival and prognostic criteria for acemannan treated HIV patients. *Antiviral research supply.* 1990; **1:** 177.
9. Wang, F., Gong, M., Chen, Y. Study on application of arbuscular mycorrhiza in growing seedlings of *Aloe vera*. *J. Chin. Med. Mat.,* 1998; **25**(1): 1-3.
10. Douglas M., Alan L. M. Nutritional Support for Wound Healing. *Alter. Med. Rev.* 2003; **8**(4): 359 -77
11. Henry, D.I. Processing and interpretation of bacterial feacal cultures. In : Essential procedures for Clinical Microbiology. *American Soc. Microbio.1985;* pp 91-94.
12. NCCLS. Performance standards for antimicrobial disk susceptibility test 3rd eds, Approved standards, National Committee for Clinical Laboratory Standards : M7 - A3,Villa Nova, P.A, 1993.
13. Oscar, M.M., Yaned, M.C., Diana, C.B., Jaime, N. Antioxidant activity of twenty five plants from Colombian biodiversity. *Mem. Inst. Oswaldo. Cruz*., 2007; **102**(5): 631-34.
14. Harborne, J.B. Phytochemical methods, A guide to modern technique of plant analysis. 3rd ed. Springer; 1998 : 1-32.
15. Ganesh, J., Larty, Y. Antimicrobial activity of *Aloe vera* and their tradicinal uses. *J. Microbio.,* 2008; **55**: 86-87.
16. Davis, R.H., Kabbani, J.M., Moro, N.P. *Aloe vera* and wound healing. *J. Amer. Paed. Med. Asso.,* 1987; **77**(4): 165-69
17. Oteo, J., Campos, J., Baquero, F. and the Spanish members of the European Antimicrobial Resistance Surveillance System. Antibiotic resistance in 1962 invasive isolates of *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. *J. Antimicrob. Chemother.* 2001; **50:** 945-52.
18. Ferro, M., Grant, F., Jones, K. I*n-vitro* susceptibility of *Shigella flexnary* and *S. pyogens* to inner gel of *Aloe barbadensis* antimicrobial agents and chemotherapeutics. *J. med*., 2003; **47:** 1137-39.