JOURNAL OF PURE AND APPLIED MICROBIOLOGY, October 2010. Vol. **4**(2), p. 853-857

Etiological Study of Diabetic Foot Ulcer Infection and Anti-foot Ulcer Activity of *Aloe barbadensis* Mill.

C. Maragatham[1] and A. Panneerselvam[2]*

[1]PG & Research Department of Microbiology and Clinical Microbiology, PRIST University (East Campus), Thanjavur, India.
[2]PG Department of Botany and Microbiology, A.V.V.M. Sri Pushpam College, Poondi, Thanjavur, India.

(Received: 13 January 2010; accepted: 20 February 2010)

Total 10 foot ulcer samples were collected from various hospitals in many places and analysed bacteriologically. Among various pathogen isolated *Pseudomonas aeruginosa* (29%) was dominant followed by *Proteus* sp., (21%) *Staphylococcus aureus* (35%), *E.coli* (7%), and *Klebsiella* sp (7%). Maintenance of sugar level in control and proper knowledge about foot care would reduced the incident of diabetic foot infection. Prompt microbiological analysis and antibiotic sensitivity profile would make the therapy more effective and reduced the progressive diabetic foot infection. The study was carried out to determine the antibacterial activity of *Aloe barbabensis* Mill. against bacterial pathogens involved in diabetic wound infection. The *Aloe barbabensis* leaf extract was prepared at varying concentration such as 25%, 50% and 100% respectively. The sterilized Muller Hinton agar were used for this diffusion study. It revealed the antibacterial activity of *Aloe barbadenisis* against *Pseudomonas* sp, *Staphylococcus aureus, Proteus* sp. and *Klebsiella* sp., but *E.coli* has resistance to *Aloe barbabensis* juice. The results showed the *Aloe barbabensis* possess significant activity against pathogenic bacteria. The above study presents only a sample of the research completed on the antidiabetic activity of *Aloe barbadensis*. The result are conclusive in each case in showing the validity of the traditional use of the *Aloe barbabensis*. Further studies are required to standardize the technology for effective utilization.

Key words: Anti microbial activity, *Aloe vera*, Bacterial culture, Foot ulcer.

Diabetes is a metabolic disorder characterized by a relatively deficiency of insulin affecting the metabolism of protein, fat, water and electrolytes. These disturbances lead to progressive and irreversible functional and structural changes in all organs of the body.

Diabetes is probably the most damaging disease which cause damage to the feet. Of the total diabetic population, 15-20% will experience a foot ulcer in their lifetime. Foot ulcers are a major predictor of future lower extremity amputations. Foot ulcers are the major complications of *Diabetes mellitus* (15%) and account for more hospitalization (20%) than any other complications. In severe cases, there is a risk of foot or partial limb amputation (50%) and occasional mortality. Diabetic ulcers are most common in forefoot beneath one of the metatarsal heads or the inter phalange joint of the cell.

Aloe barbadensis also known as *Aloe vera*. The APG II system (2003) placed the genus in the family Asphodelaceae. In the past it has also been assigned to families Aloaceae and Liliaceae or lily family.

Aloe vera leaves have a bitter, yellow latex right below the outer skin. This latex contains an anthraquinone called barbalon, which

* To whom all correspondence should be addressed.
E-mail: maragathamalagesan@yahoo.com

is activated by the intestinal flora and acts as a laxative. In its raw form, it can cause uncontrollable bowel spasms. *Aloe vera* juice is usually extracted from the whole plant and is used for chronic constipation. The juice should not be used regularly because it depletes electrolytes from the body and can cause muscle weakness.

Medicinal Value

Aloe vera has been successfully used in the healing process of burns, wounds, gastric ulcers and as a treatment of diabetes and diabetic wounds. A polysaccharide in *Aloe vera*, called "Glucomannon', works as an anti inflammatory. Aloctin A, has immune system stimulating and antitumour properties other parts have shown antiviral properties. In the present study, efforts has been made to isolate and identify the bacteria from diabetic foot ulcers and to evaluate the antibiotic sensitivity test of the bacterial isolate obtained in the study against different medicinal plants.

MATERIAL AND METHODS

Collection of samples

With the help of expert medical staff, the exudates from the foot ulcer was carefully collected in sterile moist cotton swab from diabetic patients. Various data regarding, the age, sex, occupation and associated metabolic disorders were recorded. The exudates or pus containing swabs were transported to the laboratory in a sterile container containing aimes transport media. The specimen was processed immediately within 6 hrs.

Processing of specimen

The materials collected were then smeared on slides for gram's stain. Collected pus sample was inoculated immediately to Nutrient agar plates like MacConkey agar plates and Blood agar plate, by quadrant streak method and were incubated at 37°C for 24 hrs.

Colony morphology

After incubation period, the colony characteristics of different bacterial isolates on selective plate were observed and recorded. The quadrant colonies were differentiated, then streaked on Nutrient agar slant incubated at 37°C for 24 hrs for further studies. From this, subculture was made in peptone water and incubated for 4 hours. The young culture was used for microscopic examination and various biochemical characterization.

Inoculum preparation

The common bacterial concentration for the inoculum was 10x organisms/ml. For the inoculation 2 to 4 hours old nutrient broth culture was taken with moderate turbidity. The turbidity equilavalent to that of barium sulphate standard (equilavelant to half the density (0.5) of McFarland's standard) was taken and used.

Collection of plant sample

The fresh healthy leaves of plant *Aloe barbadensis* was collected from the herbal garden of Ponnaiyah Ramajayam college of arts and science, Thanjavur. Few leaves of each plant were taken. Leaves were cleaned by washing with tap water for removal of dust and sands particles. The identification of the plant specimen was confirmed by using the standard floras (Gamble, 1997).

Agar disc diffusion method

Micro organisms are naturally susceptible to the action of specific antibiotics and are inhibited by it. The susceptibility test is done by following Bauer's method. It is the simplest method to perform the sensitivity test.

Curde leaf extract

Vincent and Vincent (1994) used modified filter paper disc method was followed filter paper disc method was followed to carry out the present investigation. About 5 gm of each leaves were weighed washed with sterilized distilled water and crushed in a mortar and pestle separately 5mm disc of Whatmann No. 1 filter paper were immersed in the filtrated leaf extracts and kept overnight. The experiment on anti-microbial activity was determined by Disc diffusion assay (Bauer *et al*., 1996) respectively.

Alcoholic leaf extract

The leaves were dried under shade and grounded to a fine texture. The leaf powder was soaked in ethyl alcohol for a week. After a week the slurry was filtered and washed to redissolved in alcohol extract. The filter rate was centrifuged for about 5 times for clarification 5 mm disc of Whatmann No.1 filter paper was immersed in the filtered alcoholic extracts and kept overnight. The experiment on anti-microbial activity was determined by Agar disc diffusion method. The discs are transferred to seeded bacterial petriplates. The plates were incubated at 37°C for

24 hours. After 24 hours the zone of inhibition was observed and measured.

Agar well difussion method

This method is commonly applied for determining the anti-microbial activity of the plant samples as well as the chemical antibiotics against the pathogens.

Preparation of plant extract

Aloe vera was taken and crushed in a motar and pestle to make 100% concentration of plant extract, water was not needed in preparing the extract usually because of the watery nature of the leaf. The concentrate was then diluted to 25%, 50% and 100% respectively with distilled water and these concentrations were used for studying anti-microbial activity.

Making of wells

The well was made in the appropriate medium with the help of well puncture. The plant extract was added to the well. Four wells were made in the each plate. The various concentration of plant juice was added to the appropriate well. The plates were then allowed to stand at room temperature for 30 minutes (per diffusion time). Then the plates were incubated at 37°C for 18-24 hours. After incubation, the plates were examined for zone of inhibition around the wells.

RESULTS AND DISCUSSION

In the current study, a total number of 10 samples were collected from various hospitals at different places to study the incidence of microorganisms in diabetic foot ulcer patients. (Table 1). The exudates from the foot ulcer patient collected from deep progressive non-healing diabetic foot ulcer patient.

Table 1. Sample collection places

S. No.	Hospitals	Locations (Tamil Nadu)
1.	Government hospital	Chidambaram
2.	Government hospital	Kumbakonam
3.	Government hospital	Mannargudi
4.	Government hospital	Mayiladuthurai
5.	Government hospital	Nagapattinam
6.	Government hospital	Pattukkottai
7.	Government hospital	Sirkali
8.	Rohini hospital	Thanjavur
9.	Government hospital	Thiruvarur
10.	KMC hospital	Trichy

Table 2. Microscopic observation of isolated pathogens

S.No.	Microorganisms	Gram's staining	Motility
1.	*Staphylococcus aureus*	Positive cocci	Negative
2.	*Pseudomonas aeruginosa*	Negative Rod	Positive
3.	*Proteus sp*	Negative Rod	Positive
4.	*Escherichila coli*	Negative Rod	Positive
5.	*Klebsiella sp*	Negative Rod	Negative

Table 3. Biochemical characteristics of isolated pathogens

S. No.	Microorganism	Indole test	MR test	VP test	Cit. test	Cat. test	Oxi. test	Coa. test	TSI test
1	*Staphylococcus aureus*	-	+	+	-	+	-	+	-
2	*Pseudomonas aeruginosa*	-	-	-	+	+	+	-	K/K
3	*Proteus sp.*	+	+	-	-	+	-	+	A/A
4	*Escherichia coli*	+	+	-	-	+	-	-	A/A
5	*Klebsiella sp.*	-	-	+	+	+	-	-	A/A

Note: +Positive, -Negative, K-Alkaline, A-Acetic. MR-Methyl red, VP- Voges-proskauer, Cit-Citrate utilization, Cat-Catalase, Oxi-Oxidase, Coa-Coagulase, TSI-Triple Sugar Iron Agar.

Microbial analysis

Colony morphology

The colony characteristics of different bacterial isolates were observed and recorded. The morphology of the organisms were observed after gram's staining and motility of the organisms were observed (Table 2).

Biochemical test

For differentiation and identification, various tests were performed. The results of biochemical tests were compared with Bergey's manual of determinative Bacteriology (Holt *et al*., 1994). The biochemical characters were represented in Table 3. The types and percentage of occurence of the pathogenic clinical isolates found in diabetic foot ulcer cases were given in Table 4.

Agar disc diffusion method

The present study describes the inhibitory effect of the crude leaf extract. Alcoholic extraction and chloroform extraction of *Aloe barbadensis* were used against five different bacteria. The anti-micriobial activity was studied by two methods namely Agar Disc Diffusion method and Agar Well Diffusion method. In this method, the anti-bacterial activity of the above mentioned leaf extract were studied by measuring the zone of inhibition diameter formed around discs. The crude leaf extract of *Aloe barbadensis* showed maximum inhibition, observed from *S. aureus* is 18mm and the minimum inhibition observed from *Klebsiella* sp is 11mm. There is no effect observed from *Proteus* sp. The chloroform extraction of *Aloe barbadensis*, showed maximum inhibition, observed from *Staphylococcus aureus* is 20mm and minimum inhibition observed from *Klebsiella* sp is 12mm. There is no effect observed from *Proteus* sp. The alcoholic extraction of *Aloe barbadensis* showed maximum inhibition, observed from *S. aureus* is 18mm and the minimum inhibition observed from *Klebsiella* sp is 10mm. There is no effect observed from *Proteus* sp. The results were recorded in the Table V. Similar observations were made by Meade *et al*., 1983; Sapico *et al*., 1984 regarding the patients having no symptoms of clinical bacteriological infection.

Agar well diffusion method

A significant zone of inhibition was seen in *S. aureus, P. aeruginosa, Proteus, Klebsiella* and no zone of inhibition was seen in *E. coli* (Table 6).

Table 4. Anti-diabetic ulcerative activity of *Aloe barbadensis* by agar well diffusion method

S. No.	Microorganism	Diameter of inhibition zone(mm)		
		25%	50%	100%
1	*Staphylococcus aureus*	10	12	20
2	*Pseudomonas aeruginosa*	20	25	35
3	*Proteus sp.*	10	15	20
4	*Escherichia coli*	R	R	R
5	*Klebsiella sp.*	10	10	17

Table 5. Percentage of bacterial isolates

S. No	Microorganism	Total number of organisms	Percentage of organism
1	*Staphylococcus aureus*	5	36
2	*Pseudomonas aeruginosa*	4	29
3	*Proteus* sp.	3	21
4	*Escherichia coli*	1	7
5	*Klebsiella sp.*	1	7

Table 6. Anti-Diabetic ulcerative activity of *Aloe barbadensis* by agar disc diffusion method

S. No.	Microorganism	Diameter of zone of inhibition		
		Crude leaf extract	Chloroform extract	Alcohol extract
1	*Staphylococcus aureus*	18mm	20mm	18mm
2	*Pseudomonas aeruginosa*	12mm	15mm	13mm
3	*Proteus sp.*	Negative	Negative	Negative
4	*Escherichia coli*	16mm	18mm	15mm
5	*Klebsiella sp.*	11mm	12mm	10mm

REFERENCES

1. Bauer, A.W., Kirby, W.M.M., Truck, H., and Shreeies, J.C., Antibiotic susceptibility testing by a standardized single disc method. *American. J. Clinical pathology*.1996; **45**(4): 493-496.
2. Gamble S., Oblendrof B., and Johnsen I, The genus hypericum a valuable resource of anti *Staphylococcul* leads. *Fitoterapia,* 1997; **73**(4): 300-304.
3. Holt, J.G., Sneath, P.H.A., Stanley, JT., and Williams, S.T., Bergey's Manual of Determinative Bacteriology. 1994; 517-551.
4. Meade, J.W., and Muller, L.B., Major infection of the foot. *Medical times*. 1983; **96**: 154-165.
5. Sapica.F., The infected foot of the diabetic patient Microbiology and analysis of Clinical features. *Review of infectious diseases*. 1984; 171-176.
6. Vincent J.G., and Vincent H.W., Filter paper disc modification of oxford cup Penicillin determination. *Proc. Soc. Exp. Med*. 1994; **55**: 162-164.
7. Angiosperm Phylogeny Group. "An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG II". *Botanical Journal of the Linnean Society*. 2003; **141**(4): 399-436.