September 2015

IJESR Volume 3, Issue 9 ISSN: 2347-6532

Aushada-Trends in translational Research

ANTIBACTERIAL EFFICACY OF *ANDROGRAPHIS PANICULATA* AND *EUPHORBIA HIRTA* AGAINST BACTERIAL PATHOGENS ISOLATED FROM DIABETIC FOOT INFECTIONS

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ABSTRACT:

The antibacterial activity of aqueous, ethanol and acetone extracts of the medicinal plants namely *Andrographispaniculata* and *Euphorbia hirta* was studied against aerobic bacteria isolated from Diabetic foot infectiions. Pus samples for the bacterial culture were collected from patients admitted with Diabetic foot infections. Gram negative Aerobic and Facultative isolates namely *Proteus mirabilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiellapneumoniae* were most frequently isolated followed by Gram positive isolates namely *Staphylococcus aureus and Staphylococcus epidermidis*. The preliminary screening of crude extracts showed good antibacterial activity against bacterial pathogens. The MIC of *Andrographispaniculata* was between 1.56mg/ml to 12.5mg/ml and for *Euphorbia hirta* it was between 3.13mg/ml to 25mg/ml. The phytochemical analysis revealed the presence of flavonoids, alkaloids, glycosides, steroids, tannins and saponins. The results suggest that a potential antibacterial drug could probably be formulated from these plant extracts to combat the effects of bacterial infections.

KEYWORDS:

Diabetic foot infections, isolation, disc diffusion, minimum inhibitory concentration, phytochemical analysis.

Introduction:

Worldwide, diabetic foot infections are a major medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes. Diabetic subjects have a relative risk up to 15-40 times greater than non-diabetic subjects to require an amputation (Nathan DM,1993)usually due to vascular, neuropathic and infectious complications. Infection with multidrug resistant organisms may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality (Hartemann-Heurtier A et al 2004). Therefore, the bacterial etiology of these wound infection and its treatment has been the focus of several studies (Bamberger DM,1987)

Therapies of bacterial infections are frequent problems due to the emergence of resistant bacterial strains to numerous antibiotics (Marimoto K et al,1989) . Some plants have shown the ability to overcome resistance in some organisms and this has led to researcher's investigating their mechanisms of action and isolating active compounds from them (Ncube NS ,2007). Nowadays, researches on medicinal plants have attracted a lot of attention globally. A number of evidences have been accumulated to demonstrate the promising potentials of medicinal plants used in various traditional, complementary and alternative systems (Kanokwan k et al ,2008).

Andrographispaniculata is an herbaceous plant, commonly known as 'king of Bitters'. Mostly the leaves have been traditionally used over the centuries for different medicinal properties in Asia and Europe as a folklore remedy for a wide spectrum of disease. The dried herb is a remedy for a number of diseases related to digestion, hepatoprotection, antibacterial, antifertility, anti-inflammatory and antityphoid activities (Matsud T ,1994) .

Euphorbia hirta commonly known as 'cat's hair' has many traditional medicinal values. This hairy plant grows up to 2 inches in height, has numerous small flowers clustered together with opposite oblong leaves. Some herbalists use it to treat dysentery and diarrhoea (Lanhers MC et al, 1994). The latex is usually applied topically to treat small wounds and also as a disinfectant (Sudhakar M et. al. 2007).

The aim of this study was to evaluate the antibacterial efficacy of the leaf extracts of two medicinal plants namely *Andrographispaniculata*and *Euphorbia hirta*against aerobic bacterial pathogens isolated from patients with Diabetic foot infections.

Materials and methods:

(i) Patients:

The pus samples for bacterial culture were collected from the patients admitted with Diabetic foot infections in Vijaya hospital, Chennai.

(ii) Clinical samples:

Clinical samples like pus and wound swabs were collected from patients using sterile cotton swabs.The clinical samples were collected using aseptic techniques to avoid contamination and were promptly sent to the laboratory and processed for aerobic bacteria. Standard methods for isolation and identification of aerobic bacteria were used (Baird v,1996)(Sutter VL,1985) . The sample collected were accompanied with Patient's history like name, age, sex, type of sample collected, date of collection etc.

(iii)Plant material:

The medicinal plants namely *Andrographispaniculata* and *Euphorbia hirta* were collected, washed with water, leaves were separated and dried for 5 days. The dried plant materials were then pulverized into coarse powder in a grinding machine (Md. Rajib Ahsan KM.2009)

(iv)Preparation of crude extracts (Chamnanpatarapanich ,2007)

The crude extracts were obtained by dissolving 10gms of plant powder in 100ml of ethanol, acetone and in 100ml of distilled water for aqueous extract and kept on a rotary shaker for 24 hrs. The extracts were filtered, then centrifuged at 5000 rpm for 15 min and was dried under reduced pressure. The crude extracts were stored at 4°C in air tight bottles. In the present

study, all preliminary phytochemical screening was carried out using the following methodologies

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(v) Antibacterial activity:

Agar disc diffusion assay:

The antibacterial activity was determined by disc diffusion method (Bauer AW,1996). . The antibacterial screening of crude extracts was done against gram positive and gram negative clinical isolates namely *Staphylococcus aureus, Staphylococcus epidermidis,Proteus mirabilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiellapneumoniae.* Each sterile disc (Himedia Disc) was loaded with 10µl (con. 100mg/ml) of test extracts and placed on the agar plates inoculated with respective microorganisms. The plates were kept for half an hour for preincubation diffusion. Then the plates were kept for incubation at 37˚C for 24hrs. The zone of inhibition (mm) was measured and recorded. Tetracycline (30µg) and Gentamicin (30µg) were used as positive control and 10% DMSO was taken as a negative control.

Determination of minimum inhibitory concentration (MIC) (Bauer AW,1996).

Micro broth dilution method was used for the determination of MIC values for each plant extract showing antibacterial activity against test pathogens. Serial dilutions of the extracts were carried out in 10 % DMSO (which has no inhibitory activity against test pathogens) to make 200 mg/ml final concentration, this was then two fold serially diluted by adding Muller-Hinton broth media in a 96 well micro titre plates to obtain 100,50,25,12.5,6.25,3.12 and 1.56µg/ml. Thereafter, 100µl of inoculum (10^8 CFU/ml) was added to each well. Bacterialsuspension was used as a positive control, while broth containing standard drug (vancomycin and gentamycin) were used as negative control. The micro titer plates were incubated at 37˚C for 24 hrs. The MIC values were taken as the lowest concentration of the extracts in the well of the micro titer plate that showed no turbidity after incubation.

Results and Conclusion:

Pus samples for the bacterial culture were collected from patients admitted with Diabetic foot infections. Gram negative Aerobic and Facultative isolates namely *Proteus mirabilis, Proteus vulgaris, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiellapneumoniae* were most frequently isolated followed by Gram positive isolates namely *Staphylococcus aureus and Staphylococcus epidermidis*.

The crude extracts of *Andrographispaniculata* and *Euphorbia hirta* was obtained by using three different solvents namely aqueous, ethanol and acetone. Thephytochemical analysis of crude extracts revealed the presence of flavonoids, alkaloids, glycosides, steroids, tannins and saponins (Table 1).

Table 1: Characterization and Identification of Phytochemicals from Crude Extracts

S.NO	CONSITUENTS	ANDROGRAPHIS			EUPHORBIA		
		AQ	E	AC	AQ	E	AC
1	Carbohydrates	-	-	-	+	-	-
2	Cardio glycosides	-	+	+	+	+	-
3	Saponins	+	+	+	+	+	-
4	Fixed oils and fats	-	-	+	+	+	+
5	Terpenoids	-	-	+	+	+	-
6	Alkaloids	+	-	-	+	+	+

7	Steroids and sterols	-	-	+	-	-	+
8	Flavonoids	+	+	+	+	+	+
9	Tannins	+	-	+	+	+	+
10	Phenolic compounds	+	+	+	+	+	+
11	Aminoacids& Proteins	+	-	-	-	-	-
12	Quinones	-	-	-	-	-	-
13	Gum	-	-	-	-	-	-

AQ - aqueous, E - Ethanol, AC - Acetone, + Positive, - Negative.

In the preliminary screening using Agar disc diffusion assay for crude extracts showed good antibacterial activity against eight bacterial pathogens. Out of two medicinal plants tested *Andrographispaniculata* showed greater inhibitory effect towards tested clinical pathogens (Table 2).

Table 2:Antibacterial activity of crude extracts against clinical pathogens

Zone of inhibition (mm)

S.NO	ORGANISM	ANDROGRAPHIS			EUPHORBIA		
		AQ	E	AC	AQ	E	AC
1	Staphylococcus aureus	12	14	10	13	15	9
2	Staphylococcus epidermidis	17	19	15	15	19	13
3	Bacillus subtilis	14	16	11	12	18	10
4	Escherichia coli	13	16	10	12	16	8
5	Klebsiellapneumoniae	15	17	13	14	17	11
6	Proteus vulgaris	12	15	9	11	14	8
7	Proteus mirabilis	10	14	6	12	14	6
8	Pseudomonas aeruginosa	8	11	4	6	9	4

The minimum inhibitory concentration of crude extracts was determined by micro broth dilution method. The minimum inhibitory concentration of six extracts were evaluated and it was found that the ethanol extracts of both *Andrographispaniculata* and *Euphorbia hirta* were effective followed by Aqueous and acetone respectively.

Among the pathogens tested *Staphylococcus epidermidis, Bacillus subtilis, Escherichia coli, Klebsiellapneumoniae, Proteus vulgaris* and *Proteus mirabilis* were the most susceptible organism towards all the crude extracts tested. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the least susceptible organism towards the extract tested. The MIC of

Andrographispaniculata was between 1.56mg/ml to 12.5mg/ml and for *Euhorbiahirta*it was between 3.13mg/ml to 25mg/ml (Table 3, Table 4).

Table 3: Minimum Inhibitory concentration of Andrographispaniculata

S.NO	ORGANISM	Concentration of the extract in mg/ml														
		25			12.5			6.25			3.12			1.56		
		AQ	E	AC	AQ	E	AC	AQ	E	AC	AQ	E	AC	AQ	E	AC
1	Staphylococcus aureus	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+
2	Staphylococcus epidermidis	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
3	Bacillus subtilis	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+
4	Escherichia coli	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
5	Klebsiella pneumoniae	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
6	Proteus vulgaris	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
7	Proteus mirabilis	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+
8	Pseudomonas aeruginosa	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+

+ indicates bacterial growth, - indicates no bacterial growth.

Table 4:

Minimum inhibitory concentration of Euphorbia hirta

S.NO	ORGANISM	CONCENTRATION OF THE EXTRACT mg/ ml				
		25	12.5	6.25	3.12	1.56

		AQ	E	AC	AQ	E	AC	AQ	E	AC	AQ	E	AC	AQ	E	AC
1	Staphylococcus aureus	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
le) 2	Staphylococcus epidermidis	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
3	Bacillus subtilis	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
4	Escherichia coli	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
5	Klebsiellapneumoniae	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
6	Proteus vulgaris	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
7	Proteus mirabilis	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
8	Pseudomonas aeruginosa	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+

From the above studies it is concluded that the traditional plants may represent new sources of antimicrobials with stable, biologically active components that can establish a scientific base for use of plants in modern medicine. These can be extended for future investigation into the field of Pharmacology, Phytochemistry, ethnobotany and other biological actions for drug discovery.

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