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ANTIBACTERIAL EFFECTS OF LEAVES AND ROOT EXTRACT OF *CALOTROPIS PROCERA* LINN.

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ABSTRACT

The analysis of antimicrobial activity of aqueous and ethanolic extract of root and leaves of *Calotropis procera* Linn, against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli* and *Pseudomonas aeruginosa* was carried out in disc method. The zone of inhibition produced by the crude ethanol and aqueous extracts against sensitive bacteria were examined. The results obtained showed that both ethanolic and aqueous extracts of *Calotropis procera* Linn, had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract of leaves and roots was significantly greater than the aqueous extract of leaves and roots. The obtained results provide a support for the use of *Calotropis procera* Linn, in traditional medicine and suggest its further advance investigation.

Keywords: Antibacterial activity, *Calotropis procera* Linn., *Escherichia coli*. *Streptococcus pyoge.*

INTRODUCTION

World over the medicinal plants are used as a main source of traditional and orthodox medicines. The attention has been made towards developing the new antibiotics that reduce the increasing resistance among the microorganism (Edith *et al.* 2005). The medicinal plants generally contain number of compounds that may be potential natural antimicrobial agents which may serve as alternative, effective, cheaper and safe antimicrobial agents for the treatment of common microbial infections (Schimmer *et al.* 1994). The use of plants extracts in medicinal treatment got a great popularity in late 1990s (Cowan, 1990). *Calotropis procera* Linn, or Milk weed or Akk belongs to family Asclepiadaceae. The family contains 280 genera and 2000 species of worldwide distribution. The most abundantly found in the tropics and sub-tropics, but rare in cold countries. *Calotropis procera* Linn, grows up to 3-6′ high, its leaves arrangement is opposite, flower size 2″ and color is white to purple, fruit is follicle (Sastry and Kavanthekar, 1990). Various pharmaceutical companies have produce a number of new antibacterial drugs in the last ten years, resistance to these drugs by bacteria has increased and has now become a global concern. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents (Nascimento *et al.* 2000). The bioactive plant extract is a new concept and has been recently reported (Aburjai *et al.* 2001).

The methanolic and aqueous extracts of leaves of *Calotropis procera* Linn, were subjected to the potential antioxidant and antibacterial activity. The results of study suggest that *Calotropis procera* Linn, stem could be a potential source of chemotherapeutic drugs for the treatment of tinea associated with epidermophyton and trichodermophyton (Kuta, 2008). The *Calotropis procera* Linn, also showed the antibacterial activity as oxytetracycline showed (Sharanu *et al.* 2007). The dyspepsia is treated by the root extract *of Calotropis procera* Linn, (Kumar *et al.* 2006). In central India its root bark and leaves are used for the treatment of jaundice (Samvatsar and Diwanj, 2000).The damages of liver by carbon tetrachloride is protected by chloroform extract of root of *Calotropis procera* Linn., (Choedon *et al.* 2006). The best method is DPPH for testing preliminary free radical scavenging activity of plant extract (Uddin,

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S.N. 2008). The ethanolic extract of leaves of *Calotropis procera* Linn, had the best antimicrobial properties (Kareem *et al.* 2008). The bacteria tested for the sensitivity were *Staphylococcus aureus, Bacillus subtilis, Eschrichia coli, Streptococcus pyogen* and *Pseudomonas aeruginosa.* The *S. aureus* is one of the most important pathogen that causes suppuration, abscess formation, a variety of pyogenic infection and even fatal septicemia in human beings. *S. aureus*, which can induce bacterimia and causes both community and hospital infections and it has developed methicillin and multi-drug resistance. *S. aureus* has become a major nosocomial pathogen (NNIS., 2004). The *E. coli* is gram-ve, non spore forming bacteria. It causes 80% urinary tract infections, gastroenteritis and miscellaneous infections (Pelczar *et al.* 1993). The *S. pyogen* is most common specie, it is beta-hemolytic and causes pharyngitis, otitis media and scarlet fever etc. This organism also produced the resistance against certain antibiotics (Gur *et al.* 2002). *P. aeruginosa* can produce infection in any tissue. It is gram-ve rod shape belongs to the family Pseudomonaceae. It causes infections especially in patients with compromised host defense mechanisms and it is the most common pathogen that isolated from patients who have been hospitalized longer than one week. It frequently causes nosocomial infections such as pneumonia, urinary tract infections and bacteremia (Pollack *et al.* 2000). The best method id DPPH (2,2-Diphenyl-1-picrylhydrazyl) for testing preliminary free radical scavenging activity of plant extracts (Uddin 2008).

MATERIALS AND METHODS

Preparation of crude extract

Fresh leaves and root of *Calotropis procera* Linn, were collected from University of Sindh, Jamshoro, Pakistan. The collection time was the month of August 2010 and plant was identified by experts of Institute of Plant Sciences, University of Sindh, Jamshoro, Pakistan. All parts were dried in the laboratory of Department Pharmacognosy, Faculty of Pharmacy, University of Sindh, Jamshoro, Pakistan, accordingly. The dried leaves and root were grinded in to fine powder by electric grinder (Sogo, china), at the speed of 500 rpm, separately. The fine powder 400 gm of each was placed in to 90% alcohol and water for maceration at room temperature for 72 hours. Then the extracts were filtered by Whatmann filter paper No: 01, respectively and were concentrated. The alcohol was removed under vacuum by rotary evaporator (349/2 J. Bibay, Science product, Limited) (Ogundiya *et al.* 2006). The crude extracts were obtained as viscous mass and kept at room temperature under the ceiling fan to get dried extract. The extracts were placed in refrigerator until use (Nazma *et al.* 2008). Some bacterial cultures were obtained from the Department of Microbiology, University of Sindh, Jamshoro, Pakistan, and some were isolated from clinical specimens cultures were identified accordingly.

Determination of antibacterial activity

The antibacterial effect was tested according to (CLSI., 2007). The method used was disc diffusion. The test was performed by using sterile 6 mm diameter paper discs (Whatmann filter paper No: 01). The discs were prepared using 10 µL of crude extract diluted to concentration of 1000, 800, 400, 200, 100 and 50 mg/mL respectively. Therefore each disc has 10, 8, 4, 2, 1, and 0.5 mg, respectively. All discs were dried at room temperature for 24 hours. Then from pure culture the organisms were transferred by wire loop to test tubes containing 5mL of Mueller-Hinton broth (MHB, Merck, E. Germany), separately. The broth cultures were incubated at 37°C for 5 hours and observed the turbidity of the 0.5 Mc Farland standard, which resulted 1 to 2 10^8 CFU/mL and mixed to pour plate, the final count was approximately 2107CFU/mL. The discs were placed on the surface of the inoculum and incubated at 37°C for 24 hours. The result was determined by measuring the diameter of the inhibition zone. Experiments were performed in triplicate and the mean of the diameter of inhibition zones were calculated.

RESULTS AND DISCUSSION

The results of antibacterial activity crude extract of *Calotropis procera* Linn., against *S. aureus*, *S. pyogen*, *E. coli* and *P. aeruginosa,* are presented in tables 1-4. The inhibition zone against *S. aureus,* ranged from 1-21mm, i.e. the aqueous and ethanolic extracts of roots showed 11, 15 mm zones of inhibition at 10 mg/disc concentration of extract and the aqueous and ethanolic extracts of leaves showed 15, 21 mm, zones of inhibition at 10mg/disc concentration of extract (Table-1). The results obtained

revealed that both extracts leaves and roots of *Calotropis procera* Linn., have bactericidal effect. Table-1 showed that the ethanolic extract of leaves has more effect on *S. aureus* as compared to aqueous extract of leaves. Ethanolic extract of roots also showed more effect on *S. aureus* than aqueous extract of roots. The inhibition zone against *S. pyogen,* ranged from 2-18 mm where the aqueous and ethanolic extracts of roots showed 11, 18mm, zones of inhibition at 10mg/disc concentration of extract where the aqueous and ethanolic extracts of leaves showed 14, 19 mm, zones of inhibition respectively at 10mg/disc concentration of extract (Table-2). The results in Table-2 showed that the ethanolic extract of leaves also has more activity against the *S. pyogen,* than the ethanolic extract of roots. The aqueous extract of leaves showed less activity as compared to ethanolic extract but it was more than the aqueous extract of roots. The results of *E. coli* inhibition zone varied from 3-18mm, i.e. the ethanolic and aqueous extracts of roots showed 14 and 15mm zones of inhibition respectively at 10mg/disc concentration of extract (Table-3). These results showed that against *E. coli,* that the aqueous extract of leaves was more active than the ethanolic extract of roots. It was also observed that the aqueous extract of leaves is more active than the alcoholic extract of roots. The zone of inhibition of *P. aeruginosa,* ranged from 1-10mm where the ethanolic and aqueous extracts of roots showed 5 and 7 mm zone of inhibition respectively at 10mg/disc concentration of extract. The ethanolic and aqueous extract of leaves showed 5.5, 10 mm zone of inhibition respectively at 10mg/disc concentration of extract (Table-4). The results in Table-4 indicated that that the aqueous extract of leaves had greater activity against *P. aeruginosa,* against ethanolic extract of roots. The aqueous extract of leaves also showed more activity against ethanolic extract of roots. Duncan's test of the antimicrobial activity of aqueous and ethanolic extracts of *Calotropis procera* Linn, revealed that had the significant effect a= 2.000, b, alpha=0.05. The effect exhibited by ethanolic extract of leaves and root was significantly more than the aqueous extract of leaves and root.

Table 1. Inhibition of *Staphylococcus aureus* by ethanolic and aqueous extracts of *Calotropis procera* Linn.

Plant part	Ethanolic extract						Aqueous extract					
Concentration mg/disc	0.5	1	2	4	8	10	0.5	1	2	4	8	10
Zone of inhibition (mm) by root	2	5	7	9	12	15	01	1.5	2	5	7.5	11
Zone of inhibition (mm) by leaves	5	8	12	15	18	21	03	5	7	10	12	15

Table 2. Inhibition of *Streptococcus pyogen* by ethanolic and aqueous extracts of Calotropis *procera* Linn.

Plant part	Ethanolic extract						Aqueous extract					
Concentration mg/disc	0.5	1	2	4	8	10	0.5	1	2	4	8	10
Zone of inhibition (mm) by root	3	6	9	13	16	18	2	3	6	9	10	11
Zone of inhibition (mm) by leaves	6	8	11	14	17	19	3	4	7	10	12	14

Table 3. Inhibition of *Escherichia coli* by ethanolic and aqueous extracts of *Calotropis procera* Linn.

Plant part	Ethanolic extract						Aqueous extract					
Concentration mg/disc	0.5	1	2	4	8	10	0.5	1	2	4	8	10
Zone of inhibition (mm) by root	3	5	7	9	11	14	5	7	9	11	13	15
Zone of inhibition (mm) by leaves	6	8	10	12	14	15	7	9	11	13	14	18

Table 4. Inhibition of *Pseudomonas aeruginosa* by ethanolic and aqueous extracts of C*alotropis procera* Linn.

Plant part	Ethanolic extract						Aqueous extract					
Concentration mg/disc	0.5	1	2	4	8	10	0.5	1	2	4	8	10
Zone of inhibition (mm) by root	1	1.5	2	3	4	5	2	1.5	4.5	6	6.5	7
Zone of inhibition (mm) by leaves	2	3	4	4	5	5.5	3	4.5	6	7	9	10

Table 5. Inhibition of *Staphylococcus aureus* by aqueous and ethanolic extracts of *Calotropis procera* Linn.

Duncan [a.b]

Conc.(mg/ disc)	N	Subset									
		Aqueous Extract.					Ethanolic extract.				
		1	2	3	4	5	1	2	3	4	5
0.5	2	3.5					2.0				
1	2		6.5				3.2	3.2			
2	2			9.5				4.5			
4	2			12.0					7.5		
8	2				15.0					9.7	
10	2					18.0					13.0
Sig		1.0	1.0	0.06	1.0	1.0	0.18	0.18	1.0	1.0	1.0

Means for groups in homogenous subsets are displayed based on Type III Sum of Squares
The error term is Mean Square (Error) = 1.083 for aqueous and 0.65 for ethanolic extract.
a. Uses Harmonic Mean Sample Size = 2.000, b. Alpha = 0.05

The inhibitory effect of *Calotropis procera* Linn, was more pronounced in the leaves than roots. Our results in accordance with Shittu *et al.* (2004). The potential activity of *Calotropis procera* Linn, in the reduction of viable count of pathogenic bacteria is quite satisfactory. The mean results were statistically analyzed using Duncan's test (Table 5-8) and means for group in homogenous subsets are displayed based on type-III sum of squares. The harmonic mean simple size is 2.000 that does not fall in critical region hence we could not reject the null hypothesis. The obtained results provide a support for the use of *Calotropis procera* Linn, in traditional medicines and suggests its further advance investigation.

Table 6. Inhibition of *streptococcus pyogen* by aqueous and ethanolic extracts of *Calotropis procera* Linn.

Duncan [a.b]

Conc.(mg/ disc)	N	Subset									
		Aqueous Extract.					Ethanolic extract.				
		1	2	3	4	5	1	2	3	4	5
0.5	2	4.5					2.5				
1	2		7.0				3.5				
2	2			10.0				6.5			
4	2				13.5				9.5		
8	2					16.5			11.0	11.0	
10	2									12.5	13.5
Sig		1.0	1.0	1.0	1.0	1.0	0.15	1.0	0.05	0.052	1.0

Means for groups in homogenous subsets are displayed based on Type III Sum of Squares
The error term is Mean Square (Error) = 0.33 for aqueous and 0.35 for ethanolic extract.
a. Uses Harmonic Mean Sample Size = 2.000, b. Alpha = 0.05

Table 7. Inhibition of *Escherichia coli* by aqueous and ethanolic extract of Calotropis *procera* Linn.

Duncan [a.b]

Conc. (mg/disc)	N	Subset											
		Aqueous Extract.						Ethanolic extract.					
		1	2	3	4	5	6	1	2	3	4	5	6
0.5	2	4.5						6.0					
1	2		6.5										
2	2			8.5					8.5				
4	2				10.5					10.0			
8	2					12.5					12.5		
10	2						14.5					13.5	16.5
Sig		1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.05	1.0	1.0	1.0

Means for groups in homogenous subsets are displayed based on Type III Sum of Squares.
The error term is Mean Square (Error) = 0.33 for aqueous extract 0.2 for ethanolic extract.
a. Uses Harmonic Mean Sample Size = 2.000, b. Alpha = 0.05.

Table 8. Inhibition of *Pseudomonas aeruginosa* by aqueous and ethanolic extract of *Calotropis procera* Linn.

Duncan [a.b]

Conc.(mg/ disc)	N	Subset							
		Aqueous Extract.				Ethanolic extract.			
		1	2	3	4	1	2	3	4
0.5	2	1.5				2.5			
1	2	2.2	2.2			3.0			
2	2		3.0	3.0			5.2		
4	2			3.5			6.5	6.5	
8	2				4.5			7.7	7.7
10	2				5.2				8.5
Sig		0.09	0.09	0.22	0.09	0.49	0.12	0.12	0.31

Means for groups in homogenous subsets are displayed based on Type III Sum of Squares.
The error term is Mean Square (Error) = 0.13 for aqueous extract 0.45 for ethanolic extract.
a. Uses Harmonic Mean Sample Size = 2.000, b. Alpha = 0.05.

CONCLUSION

The analysis of antimicrobial activity of aqueous and ethanolic extract of root and leaves of *Calotropis procera* Linn, against *Staphylococcus aureus, Streptococcus pyogen, Escherichia coli* and *Pseudomonas aeruginosa* reveals that in disc method, the zone of inhibition produced by the crude ethanol and aqueous extracts against sensitive bacteria showed both, ethanolic and aqueous extracts of *Calotropis procera* Linn and it had inhibitory effect on the growth of isolates. The effect exhibited by ethanolic extract of leaves and roots was significantly greater than the aqueous extract of leaves and roots. The results provide a support for the use of *Calotropis procera* Linn, in traditional medicine and suggest its further advance investigation.

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