ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES Knowledge to Innovation

Vol 6, Suppl 5, 2013

ISSN - 0974-2441

Research Article

IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL ANALYSIS OF *CALOTROPIS PROCERA, EICHHORNIA CRASSIPES AND DATURA INNOXIA LEAVES.*

MAHAVIR JOSHI*[2] AND SANDEEP KAUR[1]

[1]**Biotechnology Department, University Institute of Sciences, Chandigarh University, Gharuan, Mohali, Punjab, INDIA.**

[2]**Assistant Professor, Biotechnology Department, University Institute of Sciences, Chandigarh University, Gharaun, Mohali, Punjab, INDIA.**
Email: mahavirjoshi10@gmail.com

Received: 8 September 2013, Revised and Accepted: 24 September 2013

ABSTRACT

Medicinal plants are used for the formation of drugs and these plants are used traditionally to cure various diseases. These medicinal plants contain some phytochemical active compounds such as flavonoids, alkaloids, tannins, and phenols etc., which show antimicrobial activity against the pathogens. The present study investigated the antimicrobial activity of ethanol, methanol and aqueous extract of *Calotropis procera*, *Eichhornia crassipes*, and *Datura innoxia* plants, as determined on four pathogenic bacteria's i.e. *Escherichia coli (*MTCC-40), *Staphylococcus epidermidis* (MTCC-10623), *Pseudomonas aeruginosa* (MTCC- 424), and *Bacillus subtilis* (MTCC- 736) by using well diffusion method. DMSO (Dimethyl Sulfoxide) was used as solvent for plant extracts. Ampicillin and tetracycline was set as a standard and also used to compare the antimicrobial activity of plants on pathogens. Phytochemical analysis was also done for the presence of bioactive compounds. The results showed that the plants extracts of *Calotropis Calotropis procera*, *Eichhornia crassipes*, and *Datura innoxia* have potent antimicrobial activity against pathogens.

Keywords: Phytochemical, Antimicrobial, *Calotropis procera*, *Eichhornia crassipes*, *Datura innoxia,* Pathogens, Ampicillin, Tetracycline.

INTRODUCTION

Now a day most of the research work is carried out for the development of drugs from medicinal plant for the treatment of microbial and non-microbial disease [1]. Traditional use of the medicinal plants have fewer side effect over allopathic medicine such promising fact leads the development of herbal derive medicines whole over the world [2]. Plants contains flavonoids, alkaloids, Tannins, phenols etc., which have biological significance in terms of medicine development and extracts of aqueous, methanol and ethanol are good source of antiviral, antitumor and antibacterial agents [3]. *Calotropis procera* it is also known as Sodom apple. It belongs to the plant family *Asclepiadaceae*, a shrub about 6m high. Morphologically it is tall, large, branched with milky latex throughout. It is used for the treatment of various diseases in different part of the world. In India its root bark is used to cure skin diseases, enlargements of abdominal viscera and intestinal worms. In Senegal, the milky latex used to treat skin diseases [4]. *Eichhornia crassipes* also known as water hyacinth or water lily belonging to the family *Pontederiaceae* and considered as the world's most terrible aquatic weed. It has wide range of habitat ranges from tropical desert to subtropical or warm temperate desert to rainforest zones [5]. Aquatic plants such as *Eichhornia crassipes, Ipomoea aquatica and Nymphaea pubescens* that produce a variety of compounds which show therapeutic properties and can also be used as food and feed [6]. Phytochemical studies revealed that plant contains tannins, flavonoids, alkaloids and saponins. Alkaloids and flavonoids have been used as antiviral, antibacterial, antiamoebic and anticancer agents. Secondary metabolite such as phenolic and polyphenolic play important role in antimicrobial activity [7].

MATERIAL AND METHODS

Plant Material

The plants were randomly and aseptically collected from different areas of Mohali, Punjab, India. The plant materials (leaves) were washed with distilled water and dried under shadow then crushed into a fine powder with the help of grinder.

Leaves extract preparation

Plant leaves extracts were prepared using soxhlet extraction unit, a quantity of 10gm fine powder of the leaves were weighed and suspended with 200 ml of solvent. The extraction for each plant leaves carried out by using different solvent separately viz., ethanol, methanol and distilled water. The extracts were dried by using rotor evaporator, which can be store in a refrigerator at 4ºC until needed for analysis.

Working concentration

A stock solution was prepared by weighing 10 mg plant extract of each solvent viz., methanol ethanol distilled water and dissolved in 1ml of Dimethyl Sulfoxide (DMSO), in sterile eppendorf separately which results in concentration of 10,000 µg/ml. Four different concentrations ranging from 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml were prepared from the stock solution by serial dilution [8].

Test microorganisms

The pathogenic bacteria included in the present study were *Escherichia coli* (MTCC-40), *Staphylococcus epidermidis* (MTCC-10623) and the species of *Bacillus subtilis* (MTCC-736) *and Pseudomonas aeruginosa* (MTCC- 424) procured from IMTECH Chandigarh, India.

Phytochemical screening

Phytochemical analyses were done to screen phytochemical constituents by standard analytical methods [9].

Antibacterial Assay

Antibacterial activity was performed through agar well diffusion method, on nutrient agar (HiMedia) plates, spread inoculated with the test bacterial cultures. Three wells were prepared per plate with sterile cork borer, under aseptic conditions. 50 µl of test solutions was poured in wells, using micropipette. These plates were incubated at 37°C. After 24 hours of incubation, diameter of clear zones of inhibition was measured with the help of vernier caliper.

Statistical Analyses

Results are presented as mean value ± standard deviation (at least three replicate experiments).

RESULTS AND DISCUSSION

In the present investigation, results unveiled that leaves of *Calotropis procera, Eichhornia crassipes* and *Datura innoxia* have antimicrobial activity against pathogenic microorganisms. Highest percentage yield (91%) was confronted in aqueous extract of *Calotropis procera* and 32% and 39% in aqueous extract of *Eichhornia crassipes* and methanol extract of *Datura innoxia* respectively (Table-1).

Table1: It shows Percentage yield of plant leaves extract in different solvent.

Plant leaves		Dry weight Taken	Solvent volume used	Final extract weight	Percentage yield
Calotropis procera	Methanol	10g	200ml	1.9g	19%
	Ethanol	10g	200ml	0.2g	02%
	Aqueous	10g	200ml	9.1g	91%
Eichhornia crassipes	Methanol	10g	200ml	0.3g	03%
	Ethanol	10g	200ml	0.4g	04%
	Aqueous	10g	200ml	3.2g	32%
Datura innoxia	Methanol	10g	200ml	3.9g	39%
	Ethanol	10g	200ml	0.8g	08%
	Aqueous	10g	200ml	1.9g	19%

Table-2 states that standard antibiotics selected (Ampicillin and Tetracycline) showed antimicrobial activity against selected pathogens. Ampicillin showed highest zone of inhibition i.e., 32mm against *Staphylococcus epidermidis* whereas tetracycline showed 18mm against *Escherichia coli*. It was observed *Pseudomonas aeruginosa* showed resistance against standard antibiotics.

Table 2: It depicts zone of inhibition shown by standard antibiotics against pathogens.

Test organism	Zone of inhibition shown (mm) by Test organisms	
	Ampicillin (10µg/ml)	Tetracycline(10µg/ml)
Bacillus subtilis	25	12
Escherichia coli	18	18
Staphylococcus epidermidis	32	15
Pseudomonas aeruginosa	Nil	Nil

Comparative analysis from Table 3, Table-4 and Table-5 revealed that ethanol was the best solvent for extracting antimicrobial compounds from *Calotropis procera* than aqueous and methanol extract. Ethanol extract showed highest zone of inhibition at concentration of 100µg/ml against *Pseudomonas aeruginosa* whereas ethanol extraction and aqueous extract showed maximum zone of inhibition against *Escherichia coli*.

Table 3: It shows antimicrobial activity of ethanol extract of *Calotropis procera* by using agar well diffusion method.

Ethanol extract	Zone of inhibition shown (mm) by Test organisms			
conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	08±0.71	18±1.53	15±0.58	07±1
50	09±0.71	19±0.58	16±1	07±1
75	10±0.71	17±1.53	16±0.58	09±0.58
100	11±1.0	20±1.00	17±1	11±0.58

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table 4: It reveals antimicrobial activity of methanol extract of *Calotropis procera* using agar well diffusion method.

Methanol extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	06±0.58	08±0.58	11±0.58	07±1.0
50	05±1.0	09±0.58	12±0.53	08±1.0
75	08±0.58	10±1.0	11±1.0	10±0.58
100	11±0.58	11±0.58	13±0.58	14±0.58

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table 5: It shows antimicrobial activity of aqueous extract of *Calotropis procera* using agar well diffusion method.

Aqueous extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	11±1.53	07±0.57	11±1.53	11±0.57
50	10±1.0	10±1.0	12±1.0	12±1.0
75	11±0.57	12±0.57	14±0.57	14±1.53
100	14±1.0	13±0.57	14±1.53	14±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table-6, Table-7 and Table-8 state that *Pseudomonas aeruginosa* showed resistance against ethanol, methanol and aqueous extract of *Eichhornia crassipes* at standard deviation at ±0.57, ±0.57, 0±1.0 whereas ethanol extract showed maximum zone of inhibition 15±0.57 against *Escherichia coli*. Methanol and aqueous extract showed maximum zone of inhibition 15±1.0 and11±1.0 against *Bacillus subtilis*.

Table 6: It shows antimicrobial activity of ethanol extract of *Eichhornia crassipes* using well diffusion method

Ethanol extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	10±0.57	01±0.57	10±0.57	14±1.53
50	09±0.57	01±0.57	11±1.53	12±0.57
75	12±1.0	02±0.57	10±0.57	12±1.0
100	11±1.53	0±0.57	12±.0.57	15±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table 7: It represents antimicrobial activity of methanol extract of *Eichhornia crassipes* using well diffusion method.

Methanol extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	10±1.53	1±0.57	05±0.57	01±1.53
50	11±0.57	1±0.57	08±0.57	08±0.57
75	13±0.57	0±0.57	07±1.0	08±1.0
100	15±1.0	0±0.57	09±0.57	10±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table 8: It reveals antimicrobial activity of aqueous extract of *Eichhornia crassipes* using well diffusion method

Aqueous extract	Zone of inhibition shown (mm) by Test organisms

Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	09±1.53	04±0.57	04±0.57	05±1.53
50	10±0.57	01±0.57	07±1.53	07±0.57
75	11±0.57	0±1.0	08±0.57	06±1.0
100	11±1.0	0±1.0	09±1.0	09±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table-9, Table-10 and Table-11 depict that ethanol and methanol extract of *Datura innoxia* showed limited antimicrobial activity whereas the aqueous extract showed maximum zone of inhibition at 100µg/ml against *Pseudomonas aeruginosa*. Highest zone of inhibition was observed in ethanol extract of *Datura innoxia* 14±1.0 against *Escherichia coli*, Methanol and aqueous extract were showed maximum zone of inhibition of 13±0.57and13±0.57against *Escherichia coli and Bacillus subtilis* respectively.

Table 9: It shows antimicrobial activity of ethanol extract of *Datura innoxia* using well diffusion method

Ethanol extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	05±1.53	01±1.53	07±1.53	8±1.0
50	7±0.57	0±1.53	06±0.57	6±1.53
75	8±0.57	0±0.57	9±0.57	07±0.57
100	10±0.57	0±0.57	10±0.57	14±1.0

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli

Table 10: It shows antimicrobial activity of methanol extract of *Datura innoxia* using well diffusion method.

Methanol extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	10±1.0	01±1.53	07±1.53	08±1.53
50	07±1.53	0±1.53	09±0.57	10±1.0
75	05±0.57	0±0.57	08±0.57	11±0.57
100	03±0.57	0±0.57	10±0.57	13±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli.

Table 11: It depicts antimicrobial activity of aqueous extract of *Datura innoxia* using well diffusion method

Aqueous extract	Zone of inhibition shown (mm) by Test organisms			
Conc. µg/ml	*B. subtilis*	*P. aeruginosa*	*S. epidermidis*	*E. coli*
25	06±0.57	04±1.53	06±1.0	07±0.57
50	08±1.53	03±1.0	06±1.53	08±0.57
75	11±0.57	06±0.57	05±0.57	06±1.53
100	13±0.57	10±1.0	10±1.0	10±0.57

B. subtilis: Bacillus subtilis; P. aeruginosa: Pseudomonas aeruginosa; S. epidermidis: Staphylococcus epidermidis; E. coli: Escherichia coli

Phytochemical analysis results were shown the presence of various phytochemical components in plant leaves extract (Table 12).

Table12: It shows phytochemicals of *Eichhornia crassipes, Calotropis procera* and *Datura innoxia*.

Phytochemicals	*Eichhornia crassipes*			*Calotropis procera*			*Datura innoxia*		
	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+	+	_	_	+	+	+
Phenols	+	+	_	+	+	_	+	+	+
Steroids	+	+	_	+	+	+	+	_	+
Tannins	+	_	+	_	+	+	_	_	+
Terpenoids	_	+	_	_	_	+	_	_	+
Flavonoids	_	+	+	+	+	+	+	+	+

Satish *et al.,* 2007 studied the aqueous extract of *Datura stramonium,* have antifungal activity against *Aspergillus* species. They observed methanol extract was more effective than ethanol, chloroform, and benzene and petroleum ether [10]. Kaushik *et al.,* 2008 studied the antibacterial activity of *Datura innoxia* by preparing their crude aqueous and organic extracts against Gram-negative bacteria (*Escherichia coli* and *Salmonella typhi*) and Gram-positive bacteria (*Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus*). Their outcome suggested that the pattern of inhibition depends upon the part of the plant. Leaves extract were shown better activity than stem and root extracts. Their study promises an interesting future for designing a potentially active antibacterial agent from *Datura innoxia* [11].Vasu *et al.,* 2009 evaluated the *Eichhornia crassipes* produce a variety of compounds which show therapeutic properties and can be used as food and feed. For the development of new antimicrobial drugs these substances are used [6]. Vadlapudi, in 2010 concluded *Eichhornia crassipes* showed antimicrobial activities of the methanol plant extracts of against pathogens. The antimicrobial activities of the organic solvent extracts against various test microorganisms by using agar well diffusion technique [12]. Kumar *et al*., 2010 reported that Combination of secondary products such as alkaloids, steroids, tannins, and phenol compounds have medicinal effects, which are synthesized and deposited in various parts of the plant. *Datura* showed antimicrobial activity against three microorganisms *Escherichia coli, Bacillus amyloliquefaciens and Pseudomonas aeruginosa* [13]. Johnson *et al.,* 2011 screened antimicrobial property of aqueous and alcoholic leaves extract of *Datura stramonium, Calotropis procera* prepared by decoction and hot percolation process against the pathogens *Staphylococcus aureus, Escherichia coli and Aspergillus* species. Their result concluded *Datura stramonium* showed better activity against *Staphylococcus aureus* whereas *Calotropis procera* showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [14]. Baral *et al.,* 2011 studied the bioactivity of water hyacinth (*Eichhornia crassipes*) by using the soxhlet extraction (hot method) and cold percolation method in chloroform and ethanol to evaluate the antimicrobial activity of the plant. The antimicrobial activity was performed by using well diffusion method against different clinical bacterial strain. The results showed that the aquatic weed has strong antimicrobial activity and also showed the presence of biologically active phytochemicals. They concluded that water hyacinth may be useful for developing alternative compounds to treat infectious diseases caused by bacterial and fungal pathogens [15].Mako et al., 2012 analyzed the antimicrobial activity of aqueous and ethanol extract of leaves of *Calotropis procera* against *Staphylococcus aureus*, *Streptococcus pyogen*, *Escherichia coli* and *Pseudomonas aeruginosa*, by disc method. Results provide a support for the use of *Calotropis procera*, in traditional medicine and suggest its further advance investigation [16].

CONCLUSION

Antimicrobial activity of plant extract (methanol, ethanol and aqueous extract) of *Calotropis procera, Eichhornia crassipes* and *Datura innoxia* leaves was observed against pathogens viz., *Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa*. The antimicrobial activity of plant leaves extract almost shown against *Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli* but *Pseudomonas aeruginosa* show less sensitivity against all plant extract. Phytochemical analysis was also done which shows the phytochemical constitutes such as alkaloids, flavonoids and tannins are present in plants and may possesses pharmaceutical and medicinal value. Our study concluded that natural plants contain constituent that help to cure the bacterial infectious disease.

ACKNOWLEDGEMENT

Authors would like to thanks Mr. Gurdev Singh of Botany Dept. Punjab University, Chandigarh, Punjab, INDIA for identification of plant.

REFERENCES

1. Kawo AH, Mustapha A, Abdullahi BA, Rogo LD, Gaiya ZA, Kumurya AS. Phytochemical properties and antibacterial activities of the leaf and latex extracts of *Calotropis procera* (AIT.F.) AIT.F. Bajopas 2009; 2(1): 34 -40.
2. Pal SK, Shukla Y. Herbal Medicine: Current Status and the Future. Asian Pac J Cancer Prev 2003 ; 4(4):281-8.
3. Cowan MM. Plant products as antimicrobial agents. Clin Micro Rev 1999;12(4): 564–582.
4. Kareem SO, Akpan I,Ojo OP.Antimicrobial activity of Calotropis procera on selected pathogenic microorganisms. Afr J Biomed Res 2008; 11 : 105–110**.**
5. Umar, Mohammed BA. Effect of Water Hyacinth (*Eichhornia Crassipes* (Mart) Solms Leaf Extract on the Juvenile Mortality of *Meloidogyne Incognita*. J Agri Vete Sci 2013; 4(2) :46-48.
6. Vasu K, Goud JV, Suryam MA, Charya MAS. Biomolecular and phytochemical analyses of three aquatic angiosperms. Afri J Micro Res 2009; 3(8): 418-421.
7. Lattanzio V, Lattanzio VMT , Cardinali A. Role of phenolics in the resistance mechanisms of plants against fungal pathogens and insects. Phytochemistry: Adv Res 2006; 23-67.
8. Cheesbrough M. "District Laboratory practice in Tropical countries", Cambridge University Press London 2002; 2: 137-140.
9. Thamaraiselvi, Lalitha P, Jayanthi P. Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of *Eichhornia crassipes* (Mart.) Solms. Asia J Plan Sci Res 2012; 2 (2):115-122.
10. Satish S, Mohana DC, Raghavendra MP , Raveesha KA. Antifungal activity of some plant extracts against important seed borne pathogens of *Aspergillus sp*.. J Agri Tech 2007; 3(1): 109-119.
11. Kaushik P, Goyal P. "In vitro evaluation of *Datura innoxia* (thorn-apple) for potential antibacterial activity." Ind J Micro 2008; 48 (3):353-357.
12. Vadlapudi V. In vitro antimicrobial activity of methanolic extract of selected indian medicinal plants. Pharmacophore 2010; 1 (3): 214-219.
13. Kumar A, Garg BR, Rajput G, Chandel D, Muwalia D, Bala I, Singh S. Antibacterial activity and quantitative determination of protein from leaf of *Datura stramonium* and *Piper betle* plants. Pharmacophore 2010;1(3):184-195
14. Johnson DB, Shringi BN, Patidar DK., Chalichem NSS, Javvadi AK. Screening of antimicrobial activity of alcoholic & aqueous extract of some indigenous plants. Indo-Glo J Phar Sci 2011;1(2):186-193.
15. Baral B, Vaidya GS, Bhattarai N. Bioactivity and biochemical analysis of water hyacinth (*Eichhornia crassipes*). Botanica Orientalis: J Pla Sci 2012;8: 33-39.
16. Mako GA, Memon AH, Mughal AR, Pirzado AJ, Bhatti SA. Antibacterial effects of leaves and root extract of *Calotropis procera* Linn. Paki J Agri, Agri Engi Vet Sci 2012;28 (2):141-149.