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Evaluation of the effect of antimicrobial activity of ethanol extract of Calotropis *procera* in Extended Spectrum Beta- Lactamase Producing *E. coli*

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Abstract

Infectious diseases are responsible for thousands of worldwide early death .Many commercial antibiotics have been used to control human's infectious diseases all over the world. *Calotropis procera* Linn a wild growing plant of Asclepiadaecae family is known to possess multifarious medicinal properties. Total 80 non repetitive clinical *E. coli* strains recovered during 7 month, and were screened for ESBL production by disc diffusion test, and the MIC and MBC of *Calotropis procera* chlorophyll extract against ESBL positive *E. coli* isolates were determined. The results showed a total of 30 of 80 (37.5%) isolates harboured ESBL enzymes. *Calotropis procera Calotropis procera* extract were effective against ESBL producing *E. coli* isolates. There is need for a correct and reliable phenotypic test to identify ESBL beta lactamases and also these bioactive plants may help alleviate the problem of drug resistance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened using additional solvents, could yield drugs with pharmaceutical significance.

Key words: *Escherichia coli*, *Calotropis procera*, Extract, Antibiot resistant

Introduction

In this context, resistance in Gram negative bacteria presents a major challenge for the antimicrobial therapy and significantly narrows the treatment options of human infections (Maragakis, 2010). Urinary tract infection (UTI) possess a serious health threat in terms of antibiotic resistance and high recurrence rates (Lorente Garin et al., 2005). The Extended Spectrum Beta Lactamases (ESBL) producing bacteria identified in members of Enterobacteriaceae are increasingly causing urinary tract infection both in hospitalized and outpatients (Reddy et al, 2007; Dolapic and Bulteni, 2005). Microorganisms responsible for urinary tract infection (UTI) such as *E.coli* have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat (Zahar et al., 2007). *Calotropis procera* Linn . (Called Akond in Bengali Swallow wort in English; Akundia in Hindi), a wild growing plant of Asclepiadaecae family is known to possess multifarious medicinal properties. Different parts of the plant have been used in Indian traditional system of medicine for the treatment of leprosy, ulcers, tumors piles and diseases of spleen, liver and abdomen (Simbo, 2010). *Calotropis procera* is either used alone or with other herbs to treat common diseases such as fever, rheumatism, indigestion, cold, eczema and diarrhoea. In addition, combination of the latex with honey is used as antibodies and also in the treatment of toothaches and cough (Figueiredo et al., 2004). The leaf extract, chopped leaf and latex of C. procera have shown great promise as nematicides in-vitro and in-vivo condition (Babu and Uma Maheswari, 2006). The present study was conducted to evaluate

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the antibacterial activity of *Calotropis procera* plant against ESBL producing *E. coli* isolated from UTI patients.

MATERIAL AND METHODS

Isolation of E. coli: 80 strains of *E. coli* were isolated from urine culture of hospitalized patients (Amam-khomeny Hospital, Zabol, south-eastern Iran) suffered from urinary tract infections during the years 2011 and 2012. The samples were examined microscopically by Gram's stain. Samples with Gram negative results were inoculated on plates of nutrient agar, clede agar, macConkey's and blood agar then incubated at 37°c for 24 hour. The colony showed fermenting of lactose on macConkey agar and cled agar media were purified and identified according to their morphology as circular, rose - pink to red colonies on macConkey agar medium and yellow colonies on cled agar. The isolated plates were identified by biochemical reactions e.g. catalase enzyme, potassium hydroxide test, Indole and methyl red test, voges proskaur reaction, urease and citrate, H2S and oxidase test.

Phenotypic confirmatory test (PCT)

For ESBL assay, bacterial suspensions with concentration of 1.5×10^8 cfu/ml (0.5 McFarland standard) were prepared in nutrient broth. Oxoid combination disk method was used for detection of ESBLs producing organisms. In this method the bacteria were cultured on a Muller-Hinton agar plate, then ceftazidime (30 µg) and ceftriaxon (10 µg) disks (Mast, UK) were placed on media in 20-30 mm with other disks. The plates were incubated for 18-24 h at 37 °c. In this method the bacteria were cultured on Muller- Hinton agar plate, the Gentamicin, Trimethoprin-sulfametoxazol, Ciprofloxacin, Nalidixin acid, Ampicillin, Nitrofuranton, Cfecsetin disk were placed on media in 20-30 mm with other disk. The plates were incubated for 18-24 h at 37^oc.

Plant extraction:

Plant species in this study were gathered from Baluchestan region (from Sistan and Baluchestan province)were dried in natural condition and shade, then they were crushed.40 grams of powdered and dried milkweed was used to isolate extract in 500 ml Erlenmeyer flask containing 300 ml of Chloroform. The content of Erlenmeyer flask was mixed for 24 hours in room temperature with 130 rpm in a shaker and it was then filtered through watman filter paper (No.-42). Isolation of solvent from extract was performed using Rotary vacuum pump (vacuum distillation). The extracts were weighed and then dissolved in DMSO solvent and stored in 4° C till used for antimicrobial experiment.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of plant extracts:

The broth microdilution method was used to determine MIC and MBC (Zhang et al., 2013). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v/ v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranged from 0.3 mg/ml to 10.00 mg/ml. To each well, 10 µl of indicator solution (prepared by dissolving a 10-mg extract in 2 ml of DMSO) and 10 µl of Mueller Hinton Broth were added. Finally, 10 µl of bacterial suspension (10^6 CFU/ml) was added to each well to achieve the concentration of 10^4 CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18–24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of 3 values was calculated providing the MIC and MBC values for the tested extracts. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The microorganism growth was indicated by

turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

Statistical assessment:

All the experiments and measurements were repeated at least three time . All the statistical assessments were performed using SPSS and Excel 2010 software.

RESULT

Antibiotic Susceptibility:

In this study, 30 out of 80 E. coli isolates were ESBL producing organisms by disc diffusion. Antibiotic susceptibility of *E.coli* isolates was evaluated for 7 antibiotic. However, overall, *E.coli* was resistance to 7 of the agents including Gentamicin(57.31), Trimethoprin-sulfametoxazol(94.78), Ciprofloxacin(36.47), Nalidixin acid(63.52), Ampicillin(68.73), Nitrofuranton(31.26), Cfecsetin(84.36)(Table1).

Maximum and minimum of essences concentration:

The antimicrobial activity of the extract and their potency was quantitatively assessed by the presence or absence of inhibition. Plant extracts showed inhibitory activity against *ESBL- E.coli* with varying magnitudes and these effects were dose dependent manner. The levels of MIC and MBC were observed in ranges from 1.25 to 5 and 2.5 to 10 mg/ml in radius respectively (Table2) and the least MIC value was observed by the extract of *Calotropis procera* against *ESBL- Ecoli* (1.25 mg/ml).

DISCUSSION

Based on the results of this study, the prevalence of ESBL producing *E. coli* was high (37.5%).In the study of Tashkori the results of 19.86% isolated *E.coli* showed resistance to third generation cephalosporines and 10.27% were ESBL producer. In the study of Shahi, the prevalence of the ESBLs producing organisms was reported as 75% in *E. coli* isolates (Shahi et al., 2013). In the study of Badal, the prevalence of these organisms was 11.0% and 38.9% for *E. coli* and *K. pneumoniae*, respectively (Badal et al., 2013). Antibiotic resistance surveillance is necessary to determine the size of the problem and to guide the empirical selection of antibiotic agents for treating infected patients. Using appropriate antimicrobial agents and the early removal of unnecessary interventional apparatus are important for the control and decreasing the prevalence of ESBL- producing *Escherichia coli (*Lan et al., 2005). In this study the effect of the chloroformic extract of Calotropis *procera* in ESBL- E.coli was examined. The results showed that the minimal inhibitory concentration of plant extracts milkweed was 2.5 mg / ml. These results are consistent with studies of Takazxa (Kareem et al., 2008). In the study of Nenach the results showed that Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) were more susceptible than the Gram-negative (*Pseudomonas aeruginosa* and *Salmonella enteritidis*) and the yeast species were more susceptible than the filamentous fungi (Nenaah , 2013). Another study reported that crude methanol extract of *C. procera* showed antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Plesiomonas shigelloides, Shigella dysenteriae, and Vibrio cholera,* on the other hand aqueous extract showed antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus pyogenes Plesiomonas shigelloides, Shigella dysenteriae, Vibrio cholerae, Shigella Flexner, Shigella sonnei and Pseudomonas aeruginosa(*Yesmin et al., 2008*)*. Another study showed that Calotropis *procera* -protein inhibited the growth of S. aureus and E. aerogenes effectively at 25 µg/ml concentration(Perumal Samy and Chow, 2012). The results of Kawo showed that the ethanolic extracts of both the leaf and latex of C. procera have antibacterial activities on *E. coli* and *S. aureus* but with no activity against Salmonella sp and Pseudomonas sp at all concentrations. However, the antibacterial effect was more pronounced against E. coli, which was seen to be more

sensitive to both the leaf and latex ethanolic extracts at a concentration of 10,000 µg/ml with zones of inhibition of 15 mm and 10 mm respectively (Kawo et al., 2009).

CONCLUSIONS

Based on the pharmacological results of the present study, it could be said that the plant extracts contain chemical constituents with pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant, if properly screened using additional solvents, could yield drugs with pharmaceutical significance. Further research is therefore recommended to isolate, purify and characterize these chemical constituents with a view to supplementing conventional drug development especially in developing countries.

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Table 1: susceptibility pattern of E.coli strains related to different concentration of Calotropis procera (%)

10	5	2.5	1.25	0.62	
0	29.74	65	5.26	0	**MIC**
100	70.26	5.26	0	0	**MBC**

Table2: The antibiotic pattern susceptibility ESBL- E.coli (%)

Am	CF	FM	NA	CP	SXT	Gm	
26.5	57.31	10.42	6.31	42.10	5.26	21.05	**S**
5.21	57.31	57.31	50.21	52.10	78.15	36.47	**I**
68.73	**84.36**	**31.26**	**63.52**	**36.47**	**94.78**	**57.31**	**R**

Gm= Gentamicin, SXT= Trimethoprin-sulfametoxazol, CP= Ciprofloxacin, NA= Nalidixin acid, Am=Ampicillin, FM= Nitrofuranton, CF= Cfecsetin