REPORT

Antibacterial potential of *Calotropis procera* (flower) extract against various pathogens

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Abstract: Increased bacterial resistance towards commonly used antibiotics has become a debated issue all over the world in a last few decades. Due to this, consumer demand towards natural anti-microbial agents is increasing day by day. Natural anti-microbial agents have gained enormous attention as an alternative therapeutic agent in pharmaceutical industry. Current study is an effort to explore and identify a bactericidal potential of various solvent extracts of *Calotropis procera* flower. Flowers of *C. procera* were extracted with hexane, butanol, ethyl acetate and aqua to evaluate the antibacterial activity by agar well diffusion method against the various human pathogens. The microorganisms used in this study includes *Salmonella typhi*, *Escherichia coli* (O157:H7), *Micrococcus luteus* KIBGE-IB20 (Gen Bank accession: JQ250612) and methicillin resistant *Staphylococcus aureus* (MRSA) KIBGE-IB23 (Gen Bank accession: KC465400). Zones of inhibition were observed against all four pathogenic strains. Fraction soluble in hexane showed broad spectrum of inhibition against all the studied pathogens. However, fractions soluble in ethyl acetate inhibited the growth of *E. coli,* MRSA, and *M. luteus*. In case of butanol and aqueous extracts only growth of *M. luteus* was inhibited. Results revealed that the flower extracts of *C. procera* have a potential to be used as an antibacterial agent against these pathogenic organisms.

Keywords: *Calotropis procera,* antibacterial potential, human pathogens, agar well diffusion method, hexane extract.

INTRODUCTION

In the last few decades, several new natural anti-microbial compounds were discovered for the control of severe infections. A discovery of new antibacterial agents against multidrug resistant organisms is still in full swing due to the development of continuous resistance developed by microbes. The multidrug resistant organisms have received great clinical attention because of increasingly reported cases around the globe. Along with this, there is an increase consumer demand for those drugs, which are isolated or derived from natural sources. Threat posed to general public health by various multidrug resistant organisms and pathogens can be resolved by the discovery of natural antibacterial compounds having effective broad spectrum inhibition against pathogens prevalent in the local community. The anti-microbial potential of *Calotropis procera* against human pathogens was previously investigated by several researchers. *Calotropis procera* belong to the family Asclepiadaceae and commonly known as "AAK". The flower *C. procera* is widely distributed in Asia, Africa and Arab countries (Mohanraj *et al*., 2010). *C. procera* flowers (fig. 1) are arranged in terminal or axillary umbelloid cyme, consists of five deeply lobed and dirty white sepals with purple tips and white base, corona of five fleshy laterally compressed lobes surrounding the pentagonal stigma (Ali 1983). *C. procera* is medicinally very important due to its anaesthetic properties (Kawo *et al*., 2009) and its crude extracts are commonly used in traditional medicines and also in veterinary practises (Dewan *et al*., 2000; Alencar *et al*., 2004; Kareen *et al*., 2008; Johnson *et al.*, 2011). The milky sap of *C. procera* is also found to be very useful in alternative medicines (Goyal and Mathur, 2011). *C. procera* flowers are used as therapeutic agents to treat inflammation (Mascolo *et al*., 1988; Basu and Chaudhuri 1991; Neenah and Ahmed, 2011), cholera, wound, piles and asthma (Mohanraj *et al*., 2010). Sharma *et al*. (2001) and Mohanraj *et al*. (2010) also reported the use of *C. procera* as appetizer and tonic. Beside this the extracts of *C. procera* also used as an antibacterial agent against Gram's positive and Gram's negative bacteria (Mascolo *et al*., 1988; Sharma *et al.,* 2001; Parabia *et al.,* 2008; Devi *et al.,* 2008; Varahalarao and Naido, 2010; Johnson *et al*., 2011; Ahmed *et al.,* 2011; David *et al*., 2011; Doshi *et al.,* 2011; Patil and Saini, 2012). The present study is an effort to evaluate the antibacterial potential of *C. procera* using different solvent fractions of flowers with butanol, hexane, ethyl acetate and aqueous against various human

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pathogens to substantiate the earlier findings for its significant use.

MATERIALS AND METHODS

Plant materials

The fresh flowers of *Calotropis procera* were collected from natural population growing around the vicinity of Karachi during 2010-2011. Voucher specimens were deposited in the Karachi University Herbarium (G.H. No. 86455).

Extract preparation

About eight kilo-gram flowers of *C. procera* were collected and washed properly with tap water. The flowers were air dried at room temperature for one month. The dried flowers were then crushed into fine powder with the help of grinder. About 700gms of the dried flower was soaked in 80% ethanol for ten days. To obtain crude extract, the sample was filtered through a filter paper. The extract was concentrated by using Buchi Rotavapor R-200 (Buchi Labortechnik AG, Switzerland) rotary evaporator. The resulting residues were stored at 4°C until used for fractionation.

Fraction preparation

The ethanol concentrated extract was used for fractionation using separating funnel. A series of solvents were used to separate different fractions soluble in hexane, ethyl acetate and butanol. Aqueous fraction was collected during separating funnel fractionation. Fraction of hexane and ethyl acetate was concentrated on Buchi Rotavapor R-200 while butanol fraction was concentrated with the help of Eyela Rotary Vacuum Evaporator (Model No. N-10, Tokyo Rikakikai Co. Ltd. Japan). The resulting residues were then dried until it turns into solid form. The solid residue was stored at 4°C.

Indicator organisms

The anti-bacterial activity of flower extracts was determined against four human pathogenic bacterial strains. *Salmonella typhi* and *Escherichia coli* (O157:H7) were Gram's negative organisms isolated from contaminated water samples. Whereas, *Micrococcus luteus* KIBGE-IB20 (GenBank accession: JQ250612) and methicillin resistant *Staphylococcus aureus* (MRSA) KIBGE-IB23 (Gen Bank accession: KC465400) were Gram's positive organisms isolated from soil sample and clinical specimen respectively.

Culture conditions

For the revival of the culture, all the strains were grown in nutrient broth at 37°C for 24 hours with the agitation of 135 rpm. For further studies strains were maintained on nutrient agar slants at 4°C.

Anti-microbial activity assay

To determine the anti-microbial potential of flower extracts fractionated in different solvents, agar well diffusion method (Tagg and Mcgiven, 1971) was performed. Nutrient agar was poured in sterilized plates and was incubated at 37°C for 24 hours. Next day wells were punctured on nutrient agar plates previously spreaded with 100µl culture of each indicator strain containing 10^8cfu/ml compared with the 0.5 McFarland turbidity index. Concentrated fractions (100µl) were added in wells and plates were incubated at 37°C for 24 hours. Solvents without flower extracts were used as a negative control. Zones of inhibition were measured in millimeters to determine the anti-microbial activity.

The values presented in table are means of three replicate experiments with the standard deviation of ±3.

RESULTS

The Current study was designed to explore the anti-bacterial potential of medicinally important flower *C. procera* against various pathogenic as well as drug resistant organisms of our community. Different soluble flower extracts of *C. procera* showed differential spectrum of inhibition against *S. typhi*, *E. coli*, methicillin resistant *S. aureus* (MRSA) and *M. luteus* (table 1). Amongst all the extracts, hexane fraction has been proved very significant as an antibacterial agent against all the studied pathogens. Maximum zone of inhibition (22mm) was observed against *M. luteus* (fig. 1). Butanol and Aqua fractions also exhibited inhibitory activity against *M. luteus* whereas; other indicator strains were resistant to both fractions. Fraction of ethyl acetate showed inhibitory activity not only against *M. luteus* (25mm) but also against MRSA (18mm) and *E. coli* (15mm).

DISCUSSION

Resistance to different broad-spectrum antibiotic has now become a global concern due to emerging cases of drug resistance (Mohanraj *et al*., 2010). Due to these emerging cases and also due to the increase consumer demand towards natural antibacterial agents there is a need of screening of natural anti-microbial compounds effective against different drug resistant pathogens. In the last few decades; several new natural anti-microbial compounds were discovered for the control of severe infections. Keeping this in view, the present study was designed to explore the anti-bacterial potential of medicinally important flower *C. procera*.

Different soluble flower extracts of *C. procera* showed differential spectrum of inhibition against tested pathogenic organisms. Amongst all the extracts, hexane fraction has been proved very significant as an antibacterial agent against all the studied pathogens.

Maximum zone of inhibition was observed against *M. luteus* which is an opportunistic pathogen and can cause infections in immune-compromised individuals (Seifert *et*

al., 1995). It is also noteworthy that present findings are in contrast to the earlier findings (Parabia *et al*., 2008) where hexane fraction of apical twig showed least antibacterial activity (7mm) against *M. luteus.*

Fraction of ethyl acetate showed inhibitory activity not only against *M. luteus* but also against MRSA and *E. coli*, which are complementary with the previous study (Patil and Saini, 2012). *E. coli* is a toxin producing human pathogen. *E. coli* (O157:H7) is an enteric hemorrhagic strain and cause severe diarrhea leads to kidney failure through food. However, MRSA is also a potent human pathogen, involved in various hospital acquired infections and found to be resistant to all β-lactam antibiotics (Que and Moreillon, 2010; Iqbal *et al*., 2005) but in the current study ethyl acetate and hexane extracts of *C. procera* significantly inhibited the growth of this multidrug resistant organisms. Varahalarao and Naido (2010) demonstrated the antibacterial potential of extracts of *C. procera* extracted in hexane, chloroform and methanol against *Alternaria alternate*, *Aspergillus flavus*, *Aspergillus niger*, *Bipolaris bicolor*, *Curvularia lunata*, *Penicillin expansum*, *Pseudomonas marginalis* and *Rhizoctonia solani*. In another study ethanolic flower

Table 1: Antibacterial activity of flower extracts against different pathogenic strains.

Extracts	Zones of inhibition (mm)							
	Salmonella typhi	control	*Escherichia coli*	control	MRSA	control	*Micrococcus*	control
Butanol	-ve	-ve	-ve	-ve	-ve	-ve	30	-ve
Ethyl acetate	-ve	-ve	15	-ve	18	-ve	25	-ve
Aqua	-ve	-ve	-ve	-ve	-ve	-ve	30	-ve
Hexane	13	-ve	12	-ve	15	-ve	22	-ve

Key: MRSA: Methicillin resistant *Staphylococcus aureus,* Significant zone: > 11 mm, -ve: No activity detected.

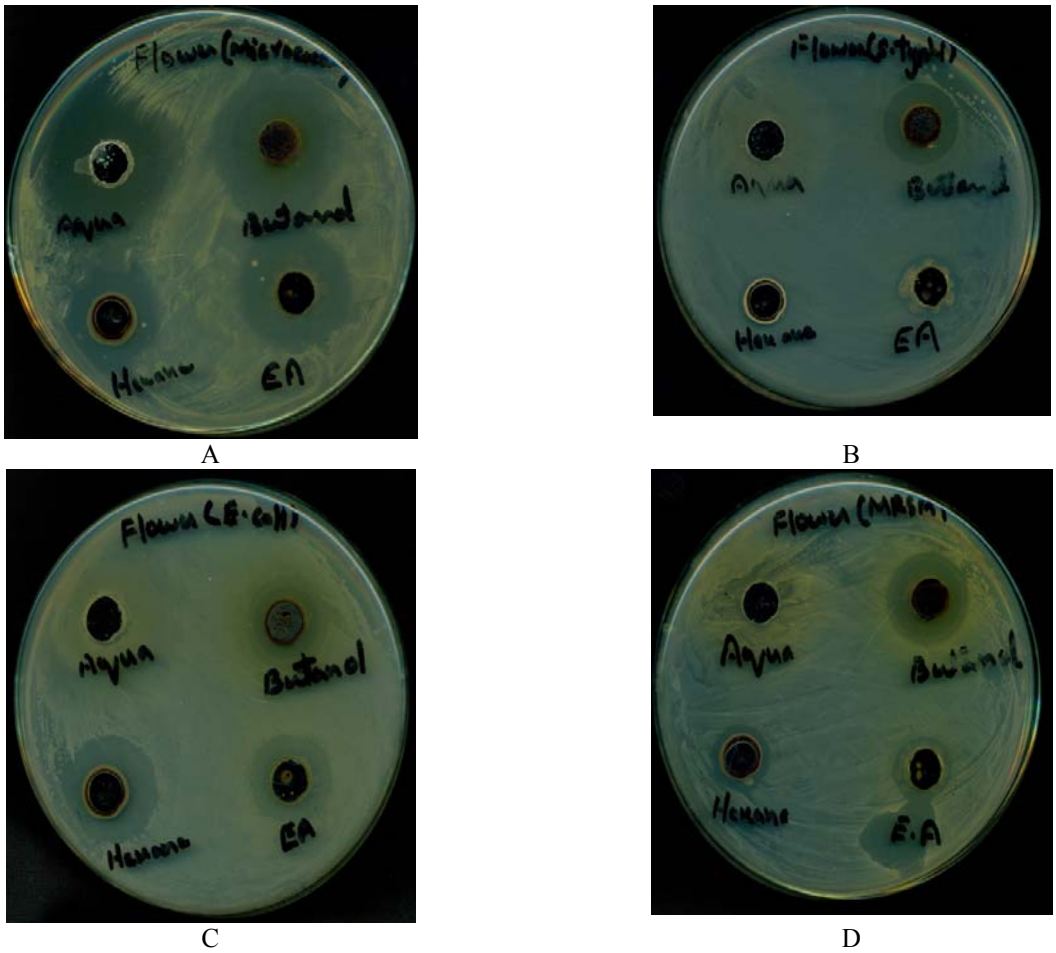

Fig. 1: Zone of inhibitions of flower extracts of *Calotropis procera* against various pathogens using agar well diffusion assay. *Micrococcus luteus* (A), Salmonella *typhi* (B), *E.coli* (C), MRSA (D).

extract was used against the larvae of *A. stephansi* (Doshi *et al.,* 2008). Parabia *et al.* (2008) used acetone, methanol, ethanol, hexane, chloroform and ethyl acetate fractions against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus cereus*, *Pseudomonas aeroginosa*, *Kleibsiella pneumonia*, *Serratia marcenes*, *Bacillus subtilis and Micrococcus luteus*. Davis (2008) reported the anti-fungal potential of water, methanol and ethyl acetate flower extracts against *Fusarium* and *T. vesiculatum*. However, acetone and methanolic flower extracts were used against *Bacillus pumilis*, *E.coli*, *A. niger*, *Fusarium oxysporum,* (David *et al*., 2011) *Salmonella* para typhi A, *Salmonella* para typhi B, *Bacillus subtilis*, *Bacillus thuringiensis*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeroginosa*, *S. aureus* and *E. coli* (Prabha *et al*., 2012).

After reviewing the antibacterial potential *C. procera* it is concluded that flower extracts of *C. procera* found to be highly effective not only against the common human pathogenic organisms of our community but also against multidrug resistant organism. In a nutshell, extracts of *C. procera* can be used to treat infections caused by aforementioned organisms after performing its characterization and clinical trials.

ACKNOWLEDGEMENT

Authors are indebted to the Dr. Iqbal Chaudhry, Director, HEJ Research Institute of Chemistry, University of Karachi, for providing facility of Eyela Rotary Vacuum Evaporator Model No. N-10, Tokyo Rikakikai Co. Ltd. Japan. This paper is a part of PhD thesis of first author.

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