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IN VITRO INHIBITION OF MDR BACTERIAL ISOLATES BY EXTRACTS OF DIFFERENT PARTS OF *CAESALPINIA PULCHERRIMA*

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ABSTRACT

Caesalpinia pulcherrima (Swartz) is a medicinal plant which is well reputed for the treatment of various infectious diseases and skin disorders. This study was designed to investigate the effect of various extracts of different parts of *C. pulcherrima* against multidrug resistant (MDR) microorganisms. Antimicrobial activity was determined using microbroth dilution method in a 96 well plates. MDR isolates (five Gram negative and one Gram positive) were tested for their susceptibility to methanol, acetone and aqueous extracts of almost all parts of *C. pulcherrima.* Moreover, all the extracts have also been evaluated against *S. aureus*ATCC 14028 and *E. coli* ATCC 8739. Minimum inhibitory concentrations (MICs) of plant extracts against MDR isolates tested were in the range of 1.0 to > 5.0 mg/mL. Extracts of pods, seeds, pod rinds and flowers showed significant antibacterial activity (MIC 1.0 - 3.5 mg/mL) as compared to extracts of other parts of the plant. The results highlight for the first time the antimicrobial properties of extracts of different parts of *C. pulcherrima* against MDR pathogens. Since pods, rinds, seeds and flowers of *C. pulcherrima* showed promising activity, they may be used as a potential source for developing more effective new antibacterial agents.

Keywords: All parts, Antibiotics, Antimicrobial activity, Medicinal plant, MIC, Resistance.

INTRODUCTION

The rates of resistance of pathogenic microorganisms to antimicrobial agents are increasing with alarming frequency. The appearance of bacterial resistance to antibiotics has consequently become a worldwide concern [1-3]. Acquisition of resistance to one antibiotic conferring resistance to a different antibiotic, to which the organism has not been exposed, is cross resistance [4]. It has been observed that organisms acquire resistance to one antibiotic may also become resistant to other antibiotic to which they have never been exposed. In general bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. WHO has indicated antibiotic resistance as a greatest threat in the effective management of infectious diseases [5, 6]. A significant potential approach to fight the resistance problem involves the discovery of new active antimicrobial substances with the potential of combating the challenge of emerging bacterial resistance [7]. In a more recent report WHO called for continued research into new drugs to combat the increasing threat of global antibiotic resistance [8, 9].

The knowledge of medicinal plants has been accumulated in the course of many centuries based on different medicinal systems such as Unani, Ayurveda, and Siddha. During the last few decades there has been a growing interest in the study of medicinal plants and their traditional use in different region of the world [10, 11]. Herbal medicines have been important source of products for the developing countries in treating general infectious

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diseases and overcome the troubles of resistance and side effects of the presently available antimicrobial agents [1]. Scientists from various fields are investigating plants as a new source of antimicrobial agents and thousands of phytochemicals are identified to have inhibitory effects on microorganisms. The medicinal, in particular antimicrobial properties of plants are attributed to the presence of bioactive components such as flavonoids, polyphenols, alkaloids and other compounds [13].

Caesalpinia pulcherrima (L.) Swartz. (syn. *Poinciana pulcherrima*) locally known as dwarf gulmohor and traditionally pride of Barbados and peacock flower, belongs to family *Caesalpanecae.* Itis an ornamental, drought resistant plant and its flowers and young seeds or pods are edible and also used as fodder [14-16]. It is reputed as an important medicinal plant in Indo-Pakistan subcontinent, Taiwan and South East Asia, Africa and tropical America [17-19]. Its various parts have been used for cure of a number of disorders including bronchitis, menoxenia, wheezing, pyrexia and malarial infection [20]. Leaves are used as anticonvulsant, anti-inflammatory, antioxidant, immune modulating and antitubercular agent [21, 22]. Stem bark is used as abortifacient, antiulcer and showed potent antiplasmodial activity [23, 24]. In Eastern Himalaya different parts of this plant are used to treat inflammation, muscular and rheumatic pain, earache and different cardiovascular diseases [25]. Anthelmintic activity of pods were reported in experimental animal [26]. Moreover, pods also showed significant antioxidant, anti inflammatory and analgesic properties [27]. Study of this folk remedy has revealed that its various parts possess antibacterial and antifungal activities [28-31]. *C. pulcherrima* contains numerous compounds including carbohydrates, tannins, flavonoids, betacyanins, diterepenoids, phenols and glycosides [32, 33].

Extensive literature survey revealed that antimicrobial activity of *C. pulcherrima* against different microorganism has been performed but in depth studies have not been carried out against multi drug resistant (MDR) organisms which are a serious issue of untreatable infections. The aim of this research was to explore antimicrobial activity of different extracts of all parts of *C. pulcherrima* against multidrug resistant (MDR) bacteria.

MATERIAL AND METHODS

Materials

C. pulcherrima was collected in August 2011 from Karachi University campus, and identified by the taxonomist of Department of Botony, University of Karachi. Its specimen (voucher No. 86546) was deposited in the herbarium of the same department.

Preparation of extracts

Fresh, undried, uncrushed parts of *C. pulcherrima*(flowers, leaves, pods, pod rinds, roots, seeds and stem) were extracted twice, successively with methanol (MET), acetone (ACE) and water (AQU) at room temperature. The methanol and acetone extracts obtained were evaporated under reduced pressure yielding their respective residues, CP-MET and CP-ACE while aqueous extracts were freeze dried to the residue CP-AQU.

Microorganism culture

Out of 350, 150 MDR bacteria were isolated from different clinical samples (urine, blood, pus, wound, throat swabs, fluid) of patients visited different hospitals of Karachi. Clinical isolates and ATCC bacteria maintained on MH (Mueller Hinton) agar were *Staphylococus aureus*, *Klebsiella pneumonia, Escherichia coli, Proteus mirabilis Pseudomonas aeruginosa, Salmonella typhi* and *Acinetobacter* spp. Standard methods were employed to assess the cultural, morphological and biochemical features. Antimicrobial activity was performed by using NCCLS standards (CLSI, 2012).

Minimum inhibitory concentration (MIC)

Antimicrobial activity of the plant extracts was performed by microbroth dilution method for determination of minimum inhibitory concentration (MIC). 18 Hours young culture was further incubated on shaking water bath for 2 hours, matched the turbidity of inoculums with 0.5 MacFarland standard suspension then further diluted to make 10^6 colony forming unit. Two fold serial dilution of extracts and antibiotics were prepared in Mueller-Hinton broth in microtiter plate. 20 µL of inoculums was added in each wall of microtiter plate so that total volume in each well was each equal to 200 µL. Plates were incubated at 37^0C for 24 hours to check the turbidity. The lowest dilution with no turbidity was considered as the MIC. Antibiotics were used for positive control and DMSO was taken as a negative control. Ciprofloxocin and cefurixime were used as standard antibiotics in present study.

RESULTS AND DISCUSSION

Multi drug resistant bacterial infections account for major part of the global infectious disease burden along with high mortality and morbidity. For the last twenty years, the progress of drug resistance as well as the emergence of side effects of certain antibiotics has led to the discovery of new antimicrobial agents generally among plant extracts with the target to find out new substance, which overcome the above disadvantages [34]. Plant extracts have been utilized for centuries for human health especially for wound healing and in the treatment of common infectious diseases, and due to their curative potential they have been investigated for the development of novel drugs. WHO in its 1997 guidelines stated that useful available plants locally, be used as a substitute for drugs [35]. Research work on medicinal plants and exchange of information obtained will go a long way in scientific exploration of medicinal plants for the benefits of man and is likely to decrease dependence on imported drugs [36].

In the present study antimicrobial activity of the methanol, acetone and aqueous extracts of various parts of *C. pulcherrima*, were tested against MDR organisms, one Gram-positive and six Gram-negative bacteria including *S. aureus, E. coli, K. pneumonia, P. aeruginosa, Acinetobacter* spp, *P.mirabilis*, and *S. typhi*. All the extracts exhibited good growth inhibitory activity (1>5 mg/mL) against both Gram -positive and Gram- negative clinical MDR bacteria. However, best results were shown by flower and fresh and dry pods (1-3.5 mg/mL), therefore pods were further divided into seeds and rinds, which displayed outstanding antimicrobial activity as shown in (Table I). It is important to mention that MIC values < 8 mg/mL of crude extracts are considered to be good activity [37]. Methanol extract of seeds of both fresh and dry pod showed promising antimicrobial activity (MIC 1.0 mg/mL) against MDR *E.coli*, *S.typhi* and *S.aureus*. Similarly rinds of fresh and dry pods also exhibited the same MIC (1.0 mg/mL) against *E.coli* and *K.pneumoniae.* Methanol extract of dry pod also exhibited low MIC (1.25 mg/mL) against *E.coli* (Table I). Virulent strains of *E.coli* cause urinary tract infection, gastroenteritis and neonatal meningitis. In some cases it is a cause of hemolytic uremic syndrome [38]. Methanol and aqueous extracts of fresh pod exhibited MIC of 1.25 mg/mL against *K.pneumoniae* and *S.aureus*, earlier it has been reported that chloroform, n-butanol and aqueous extracts of plant possessed good antimicrobial activity against *Methicillin resistant S. aureus* (MRSA) [39]. MRSA is a serious MDR pathogen which is a cause of skin infections, pneumonia, bacterimia and surgical site infections. It creates huge problems in hospitals, nursing homes and prisons where patients with persistent devices, open wounds and weakened immune systems are at greatest risk of infection than the common community [40]. Our research supports the use of this plant in skin infections. It has been reported earlier that alcoholic extract of pods possessed significant antimicrobial activity against non MDR *E. coli, P. aerugonisa, B. subtilis, S. aureus*and *R. oligosporous* [41]. Antimicrobial activity of methanolic extracts of seeds and rind of fruit/pods in the range of 78-5000 µg/mL, has also been reported against non MDR *S. epidermidis, B. cereus, B. subtilis, S. aureus, C. rubrum, K. pneumonia, P. vulgaris, S. typhimurium, P. aeruginosa* [42].

Moreover, methanolic and acetone extracts of roots showed good antimicrobial activity against MDR organisms, *K. pneumoniae, S. aureus, P. aeruginosa* and *S. typhi* (MIC 3.50 mg/mL) as depicted in Table I. *K. pneumoniae* causes destructive changes in human lungs and leads to hemorrhagic conditions. *Klebsiella* infections are mostly hospital acquired in people with weak immune system. The most common infection outside the hospital is pneumonia, in the form of bronchopneumonia and bronchitis [43]. Earlier methanolic extracts of roots were shown to possess good antimicrobial activity against resistant bacteria, *P. aeruginosa, S. epidermidis, K.pneumoniae* and *S. aureus* [44]. Morever, significant antimicrobial activity of roots of *C. pulchiremma* has been reported [45], while cassane furanditerpenoids isolated from the roots showed strong antitubercular activity with MIC of 6.25 µg/mL [46].

In the current study, methanol extracts of stem showed MIC of 2.5 mg/mL against MDR *K. pneumoniae* and *P. aeruginosa* (Table I). *P. aeruginosa* causes life threatening infections especially in immune compromised patients, it typically infects pulmonary and urinary tracts [47]. In literature it has been reported that ethanol and aqueous extracts of stem showed antimicrobial activity in the range of 6.25-100 mg/mL against non MDR *E.coli, S.aureus, P.aeruginosa, Proteus, K. pneumonia* and *S.typhi* (Ogu*et al*., 2010). Whereas in one study, a terpenoid 3-oxo-(20S,24S)-epoxy demmarane-19,25-diacetate isolated from chloroform extract of stem bark exhibited MIC value of 16 µg/mL and 32 µg/mL against *B. cereus* and *S. dysenteriae* respectively. Furthermore, lathyrol-3-phenyleacetate-5,15-diacetate isolated from stem bark showed significant antimicrobial activity of 64 µg/mL and 32 µg/mL against non MDR *B. cereus* and *S.dysenteriae* respectively.

In the present study, methanol and acetone extracts of flower showed low MIC of 1.25 mg/mL against MDR *P. aeruginosa*, *P. mirabilis* and *K. pneumonia* (Table I). In literature MIC of ethanol extracts of flower has been reported in the range of 2.5 -15 mg/mL against non MDR *B. subtilis, S. aureus, E. faecalis, E. coli, P. aeruginosa* and *K. pneumonia* [48]. Another report described that aqueous and ethanolic extracts of flower were effective against non MDR *E.coli, B. subtilis* and *S.aureus* with MIC in the range of 14.4 -16.7 µg/mL. Ethanolic extract of the flowers was found to be more effective against *E .coli* and *B.subtilis* than aqueous extract with MIC 14.4 -16.3 µg/mL [49]. Nagaraj *et al*. reported good antimicrobial activity of gold nano particles of flower extract against *E.coli* and *Streptobacillus* spp, as compared to standard antibiotic.

In the current study, acetone and aqueous extracts of leaves showed low MIC of 1.8 mg/mL against MDR *P.mirabilis* (Table I). It has been reported earlier that methanolic extract of leaves showed greater activity against *Klebsiella.* spp, while cassane-type furanoditerpenoids isolated from the leaves showed significant antimicrobial activity against *S.aureus, E.coli, P.aeruginosa, B.subtilis*and fungi [50]. Two new homoisoflavonoids from aerial part of plant exhibited significant antimicrobial activity against Gram-positive and Gram-negative bacteria. Present work demonstrated promising antimicrobial activity of *C. pulcherrima* against six MDR Gram-negative and one Gram-positive organisms. Normally Gram-positive bacteria are easy to kill as compared to Gram-negative organisms because they have complex cell wall structure. It has been shown that outer membrane of Gram-negative bacteria restricted the diffusion of antibiotics and plant extracts due to its thick murein layer and periplasmic space. Multidrug efflux pumps would also pump out the antibacterial agents through the active efflux processes which would create a high intrinsic resistance for Gram-negative bacteria. The results of current study indicated that all tested extracts of *C. pulcherrima* exhibited broad spectrum inhibitory effects against all the highly resistant Gram-negative as well as Gram-positive bacteria. As compared to acetone and aqueous extracts, methanol extracts were found to show much stronger inhibitory activity against MDR bacteria (Table I), as observed earlier [4]. In our study pods and flower extracts of the *C. pulcherrima* emerged as the potent antibacterial agents. On dividing the pods into the seeds and rinds the activity further enhanced in their respective methanol extracts, as observed earlier in case of sensitive bacteria [4]. Determination of chemical composition of extracts of this plant is beyond the scope of the present study. However, it has been reported that this plant is a rich source of polyphenols, *e.g* flavonoids and phenolic acids. Quercetin, gallic acid, methyl and ethyl gallates and catechin which are found in its various parts [30] may be responsible for the activity noted in its different extracts in the present investigation as they are well mentioned in literature for their antimicrobial properties [50-54].

Table 1. Minimum Inhibitory Concentration (MIC) values (mg/mL) of extracts of different parts of *C.pulcherrima* against seven MDR bacteria (1-7) and two non MDR,ATCC pathogens (8,9)

S#	Extracts	*1*	*2*	*3*	*4*	*5*	*6*	*7*	8	9
1	**ROOTS (MET)**[a]	>5	5	3.5	2	5	>5	5	5	5
2	**ROOTS (ACE)**[b]	3.5	3.5	>5	3.5	3.5	5	>5	5	2.5
3	**ROOTS (AQU)**[c]	5	>5	3.5	3.5	5	>5	5	5	3.5
4	**STEM (MET)**	>5	2.5	>5	5	2.5	>5	>5	5	5
5	**STEM (ACE)**	>5	5	5	5	3.5	>5	3.5	5	5
6	**STEM (AQU)**	5	3.5	5	5	5	>5	3.5	>5	5
7	**LEAVES (MET)**	3.5	3.5	5	5	2.5	5	>5	>5	3.5
8	**LEAVES (ACE)**	5	3.5	2.5	2.5	5	>5	1.8	3.5	3.5
9	**LEAVES (AQU)**	5	2.5	5	5	5	>5	1.8	5	3.5
10	**FLOWER(MET)**	2	2.5	1.8	2.5	1.25	2.5	1.25	2.5	3.5
11	**FLOWER(ACE)**	2.5	1.25	2.5	3.5	2.5	3.5	2.5	3.5	1.5
12	**FLOWER(AQU)**	2.5	1.8	3.12	2.5	1.25	2.5	2.5	3.5	1.5
13	**FRESH POD (MET)**	2	1.25	1.25	1.56	1.8	2	3.5	2	1.25
14	**FRESH POD (ACE)**	2	2.5	2	1.5	2	2	3.5	1.56	1.25
15	**FRESH POD (AQU)**	1.8	1.25	2	2	1.8	2.5	2.5	1.56	2
16	**FRESH POD RIND (MET)**	1.25	1	1.56	3.5	1	1.25	1.25	2.5	1.8
17	**FRESH POD RIND (ACE)**	1.56	1.25	3.5	2.5	1.25	2.5	1.25	2.5	1.56
18	**FRESH POD RIND (AQU)**	1.25	1	3.5	1	1.25	1.56	1.25	1.8	1
19	**FRESH POD SEEDS (MET)**	1	3.5	3.5	1.56	1.8	1.8	3.5	3.5	1
20	**FRESH POD SEEDS (ACE)**	2.5	1.8	1.8	1.56	3.5	2	2	2.5	2
21	**FRESH POD SEEDS (AQU)**	2.5	1.56	1.56	3.12	2.5	3.5	3.5	1.2	2
22	**DRY POD (MET)**	1.25	1.8	1.25	2.5	3.5	2.5	2.5	1.25	1.8
23	**DRY POD (ACE)**	2.5	2.5	1.25	7.5	3.5	5	5	1.25	1.8
24	**DRY POD (AQU)**	2.5	1.25	2.5	1.25	3.25	5	5	1.8	2.5
25	**DRY PODS RIND (MET)**	1	3.25	1.5	1.25	3.25	3.25	1.25	1.5	1
26	**DRY PODS RIND (ACE)**	1.25	3.25	2.5	2.5	1.5	3.25	2.5	1.8	1.5
27	**DRY PODS RIND (AQU)**	2.5	1.8	1.25	2.5	3.25	1.56	1.25	1.25	1.25
28	**DRY PODS SEED (MET)**	3.25	1.56	1	1	1.56	3.12	3.12	1.25	2.5
29	**DRY PODS SEED (ACE)**	1.56	3.12	1.25	1.56	3.12	1.56	3.12	1.25	1.56
30	**DRY PODS SEED (AQU)**	2.5	1.25	1.25	1.5	3.12	1.56	1.56	1.25	2.5
31	**Cefurixime**	0.05	>0.3	150	0.0018	>0.30	0.25	>0.3	0.002	0.003
32	**Ciprofloxacin**	0.05	0.15	>0.3	>0.3	0.15	0.10	0.25	0.0007	0.0005

[a]MET, Methanol; [b]ACE, Acetone; [c]AQU, Aqueous
1=*E.coli*, ***2***=*K.pneumoniae*, **3**=*S.aureus*, **4**=*S.typhi*, **5**=*P.aeruginosa*, ***6***=*Acinetobacter*spp, **7**=*P.mirabilus*,
8=*S.aureus* ATCC 14028, **9**= *E.coli* ATCC 87

CONCLUSION

Multiple drug resistance in microorganisms is an alarming problem throughout the world. There is an urgent need to develop new antimicrobial agents to combat infectious diseases due to resistant pathogens. In the current study extracts of different parts of *C. pulcherrima* an easily grown, edible plant were found to be active against MDR pathogens. Its flowers, pods, seeds and rinds which are abundantly available have stronger antimicrobial activity effect. The inhibitory activity of the extracts justified the medicinal use of the plant for the cure of infectious diseases. Further work is needed to explore active constituents for standardization and to carry out studies to determine mechanism of action and their synergistic combination with those antibiotics which have lost their efficacy so that novel combinations of drug can be achieved.

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