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Research Article

Comparative Antibacterial Efficacy of *Swertia chirata* and *Colocasia esculenta*

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ABSTRACT

The worldwide increase of multidrug resistance has impaired the current antimicrobial therapy and warranting the search for other alternatives. The present study was aimed to find out the antibacterial potential of *Swertia chirata* and *Colocasia esculenta* against five bacterial strains, viz. *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Escherichia coli*. Both the plants *S. chirata* and *C. esculenta* exhibit considerable antibacterial activity against all the tested microorganisms at a Minimum Inhibitory Concentration (MIC) ranging from 0.075 to 0.600 mg/ml. The results obtained from the present study reveal that *S. chirata* showed maximum inhibition against the following four bacterial strains, *P. aeroginosa*, *K. pneumonia*, *S. aureus* and *E. faecalis* at the lowest MIC value of 0.075 mg/ml. Whereas, *C. esculenta* showed inhibition only against the following two bacterial strains, *P. aeroginosa and K. pneumonia* at the same MIC value of 0.075 mg/ml. The results were also compared with a reference drug, ampicillin. The results clearly indicate that the antibacterial efficacy recorded for *S. chirata* was for a wider range of microbes than *C. esculenta*. Hence, it could be developed as a admirable broad spectrum antimicrobial agent. Conclusively, the results obtained seem to be promising showing the potentiality of *S. chirata* and *C. esculenta* in the treatment of various bacterial infections and hence both the plants could be explored for commercialisation.

Keywords: *S. chirata*, *C. esculenta*, Antibacterial, Minimum Inhibitory Concentration

INTRODUCTION

Antibiotics are one of the most important weapons for fighting bacterial infections and have played a pivotal role in improving the quality of human life since their introduction[1]. However, in recent years, many commonly used antibiotics are proving to be less effective due to emergence of antibiotic resistance[2]. Antibiotic resistance is the ability of bacteria and other microorganisms to resist the effects of an antibiotic to which they were once sensitive. The spread of antibiotic resistance as well as the evolution of new strains of disease causing agents is of great concern to global health[3]. Hence, it is imperative, to discover new drugs for infectious diseases with lesser or no bacterial resistance at all. Since, plants are rich in a wide variety of secondary metabolites, therefore, the use of herbal medicines as alternative therapy for infectious diseases has been intensified due to their high content of antimicrobial agents such as polyphenols, i.e. flavonoids, tannins, alkaloids and terpenoids[4,5]. Drugs derived from natural sources play a significant role in the prevention and treatment of various human diseases. Herbs are widely exploited in traditional medicine and their curative potentials are well documented. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious diseases[6]. In traditional medicine too, plants have been used since ancient times for prevention from infectious diseases. Thus, the modern system of medicine is becoming increasingly receptive to the use of antimicrobial and other drugs derived from plants, as most of the existing antibiotics have become ineffective due to enhanced drug resistance[7]. Another driving factor for the renewed interest in plant antimicrobials in the past 20 years has been the rapid rate of plant species extinction[8]. Hence, it is all the more necessary that the impressive array of knowledge assembled by indigenous people about plants, should be explored scientifically to be of continued use in our global health campaign[9]. In the present study two indian medicinal plants viz. *Swertia chirata* and *Colocasia esculenta* have been selected for validating their antibacterial efficacy scientifically against five bacterial strains, viz. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*.

MATERIALS AND METHODS

Plant collection, identification and extract preparation

Fresh stems (1 Kg) of *S. chirata* (Gentianaceae) and corms (1 Kg) of *C. esculenta* (Araceae) were purchased from the local market of Allahabad, U. P., India and got identified by Prof. Satya Narayan, Taxonomist, Department of Botany, University of Allahabad, Allahabad, India. A voucher specimen has been submitted to the University

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herbarium. The dried stems of *S. chirata* were soaked in distilled water and were regularly shaken for two days at room temperature. Dried corms of *C. esculenta* were boiled in distilled water for 48 hours. Both the extracts of cold and hot stems and corms respectively, were filtered and the filtrate was concentrated and dried in lyophilizer to obtain dry powder. The finally prepared *S. chirata* stems extract powder was a dark green solid material (3.8g) and *C. esculenta* corms extract powder was a dark brown solid material (9.7g). These powders were dissolved in distilled water and used for evaluation of their antibacterial activity.

Bacterial strains, stocks and growth in vitro

Bacterial strains of *Escherichia coli* ATCC 25922 (Gram-negative), *Klebsiella pneumoniae* ATCC 13883 (Gram-negative), *Pseudomonas aeruginosa* ATTC 27853 (Gram-negative), *Staphylococcus aureus* ATCC 25923 (Gram-positive) and *Enterococcus faecalis* NCIM 2923 (Gram-positive) were clinical isolates obtained from the Department of Biotechnology, All India Institute of Medical Sciences (AIIMS), New Delhi, India and the microbiologist of the department confirmed the identity based on microscopic examination, Gram's character, and biochemical test profile. Bacterial stocks were maintained and stored as 1 ml aliquots at -80ºC in Luria Bertani (LB) broth for all the five bacterial strains. Bacterial stocks were revived from -80ºC and grown in Luria Bertani (LB) broth for *E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and *E. faecalis.* All cultures were grown overnight at 37ºC ± 0.5°C, pH 7.4 in a shaker incubator (190-220 rpm). Their sensitivity to the standard antibiotic, Ampicillin was also checked. Luria Bertani broth (Himedia) and standard antibiotic, Ampicillin (Himedia) were used in antimicrobial sensitivity testing.

Determination of Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentration (MIC) for the freshly prepared inocula of *E. coli, K. pneumoniae, P. aeruginosa, S. aureus* and *E. faecalis* was determined by the micro-dilution method, using serially diluted (2-fold) extracts of both the plants, according to the National Committee for Clinical Laboratory Standards[10]. Varied concentrations ranging from 0.075 to 2.40 mg/ml were prepared from 50 mg/ml stock solution for both the extracts. The results were compared with the same concentration range of a standard antibiotic, ampicillin. The working solution in each tube was of 1 ml containing 50 µl of bacterial strain and calculated amount of broth and various concentrations of both the aqueous extracts. The test tubes closed with cotton plugs were incubated at 37ºC for 24 h in a shaker incubator. The lowest concentration of the extracts at which the turbidity disappeared was supposed to be the MIC for that particular bacterial strain. Control tubes without the tested extracts were assayed simultaneously. All samples were tested in triplicates.

RESULTS AND DISCUSSION

Results of antibacterial efficacy of *S. chirata* stem and *C. esculenta* corm based on micro-dilution method, have been shown in Tables 1 and 2, respectively. Table 1 represents the comparative MIC assessment of *S. Chirata* stem and standard antibiotic, ampicillin against five different bacterial strains. The results of Table 1 clearly reveal that aqueous extract of *S. chirata* stems exhibit considerable antimicrobial activity against all the tested microorganisms at a concentration range from 0.075 mg/ml to 0.30 mg/ml. However, it showed inhibition against the following four bacterial strains, *P. aeroginosa, K. pneumonia, S. aureus* and *E. faecalis* at the lowest MIC value of 0.075 mg/ml. Whereas, higher MIC value of 0.300 mg/ml was observed against *E. coli* strain. Moreover, results of ampicillin taken as a standard antibiotic clearly reveal that it has higher MIC values than *S. chirata* for the same strains, for example 0.150 mg/ml for *K. pneumonia, P.* aeruginosa, *E. faecalis* and 0.300 mg/ml for *S. aureus*. It is interesting to note that MIC value of standard antibiotic, ampicillin was much lesser (0.075 mg/ml) in comparison to *S. chirata* which showed minimum antibacterial activity at four times higher MIC value (0.300 mg/ml) against *E. coli*.

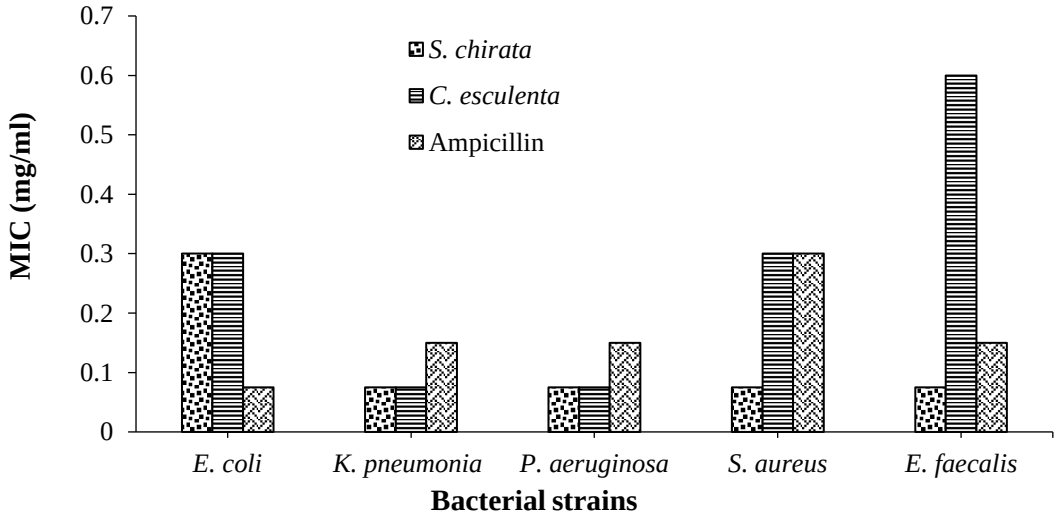

Figure 1: Graphical presentation of MIC values of *S. chirata*, *C. esculenta* and ampicillin

Table 1: MIC values of *S. chirata* stems and standard drug, Ampicilin

Microorganism	MIC (mg/ml)	
	S. chirata Stems	Standard drug Ampicilin
Escherichia coli	0.300	0.075
Klebsiella pneumonia	0.075	0.150
Pseudomonas aeruginosa	0.075	0.150
Staphylococcus aureus	0.075	0.300
Enterococcus faecalis	0.075	0.150

Table 2: MIC values of *C. esculenta* corm and Standard drug, Ampicilin

Microorganism	MIC (mg/ml)	
	C. esculenta Corm	Standard drug Ampicilin
Escherichia coli	0.300	0.075
Klebsiella pneumonia	0.075	0.150
Pseudomonas aeruginosa	0.075	0.150
Staphylococcus aureus	0.300	0.300
Enterococcus faecalis	0.600	0.150

Table 2 represents the comparative MIC assessment of *C. esculenta* corm and standard antibiotic, ampicillin, against five different bacterial strains. In case of aqueous extract of *C. esculenta* corm, antibacterial activity against all the tested organisms was observed at a concentration range from 0.075 mg/ml to 0.600 mg/ml. However, it showed inhibition against the bacterial strain, *P. aeroginosa and K. pneumonia* at the lowest MIC values of 0.075 mg/ml. Whereas, the standard drug, ampicillin, showed inhibition at just double of this concentration showing higher MIC values of 0.150 mg/ml against the same bacterial strain. Moreover, the next higher MIC value of 0.300 mg/ml was observed against *S. aureus* and *E. coli*. Though, the standard drug, ampicillin also showed the maximum inhibition at 0.300 mg/ml for *S. aureus* but its MIC value for *E. coli* was much less at 0.075 mg/ml. It clearly indicates that the antibacterial activity of both *C. esculenta* and standard antibiotic, ampicillin against *S. aureus* is same. *C. esculenta* showed highest MIC value of 0.600 mg/ml against the remaining bacterial strain, viz. *E. faecalis*. The comparative study of MIC values of *C. esculenta* and standard antibiotic, ampicillin for *E. faecalis* clearly shows that ampicillin has much lesser MIC value of 0.150 in comparison to *C. esculenta*. Thus, the results suggest that aqueous extract of *C. esculenta* has greater antibacterial activity than ampicillin against *P. aeroginosa*, *K. pneumonia* and *S. aureus* and hence it could be developed as a novel antibiotic agent for these strains with improved efficacy.

Figure 1 reveals the MIC values of *S. chirata*, *C. esculenta* and standard antibiotic, ampicillin against the five different bacterial strains. Bacterial strains *K. pneumoniae* and *P. aeroginosa* were inhibited significantly by the aqueous extracts of both the plants viz. *S. chirata* stems and *C. esculenta* corm at MIC value of 0.075 mg/ml. These results were better than even reference drug, ampicillin having MIC value of 0.150 mg/ml for the same strains. Moreover, bacterial strains *S. aureus* and *E. faecalis* both were inhibited, better than reference drug, ampicillin only by the aqueous extract of *S. chirata*, exhibiting excellent antibacterial activity at MIC 0.075 mg/ml. Whereas, *C. esculenta* corm showed inhibition at par with refernce drug, ampicillin against *S. aureus* and inhibition at higher MIC value of 0.300 mg/ml against *E. faecalis*. In case of *E. coli* both the extracts of *S. chirata* stem and *C. esculenta* corm showed identical MIC values at 0.300 mg/ml which was higher than that of the reference drug, ampicillin having MIC value at 0.075 mg/ml. Hence, the overall antimicrobial efficacy recorded for both the plants was identical against all the three bacterial strains except *S. aureus* and *E. faecalis*. The MIC values for all the five bacterial strains were further confirmed by subjecting the strains to even lower concentrations, which showed no further inhibition.

CONCLUSION

Conclusively, it could be stated that both the plant extracts have great potential to be developed as antimicrobial agents. They can be used even in the treatment of infectious diseases caused by resistant microbes. This probably explains the use of these plants by indigenous people against a number of infections since generations. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative treatment for infections. Results reveal that these plant extracts could be explored as antimicrobial agents even at a very low concentration, thus minimizing the possible toxic effects to overpower bacteria resistance which is becoming a threat to human health.

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