

Invitro Evaluation of Antimicrobial Activity of the plant extracts of *Elytraria acaulis*

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ABSTRACT

Antimicrobial activity of the aerial parts of the *Elytraria acaulis* a stem less perennial herb of *Acantheaceae* family has been carried out in the present study. Extracts of the aerial parts of the plant (Stem & Leaves) were prepared in different organic solvents such as n-Hexane, Ethanol, Methanol and Ethyl acetate. All the extracts were analyzed for antimicrobial properties against various pathogenic bacterial infections (*Escherichia coli*, *Klebsiella Species*, and *Salmonella Typhi*). Amongst all the extracts, methanolic extract exhibited significant antimicrobial activity. The crude methanol extracts of leaves showed good inhibitory effects against pathogens. Hence further studies on this plant will enable elucidation of its therapeutic properties and medicinal applications.

Keywords: Aerial parts, Antimicrobial activities, *Elytraria acaulis*, Organic solvents, Pathogenic bacterial infections.

I. INTRODUCTION

Anti-microbial substances are biological, semi-synthetic/synthetic origin produced by fungus/ bacterium or by medicinal herbs as secondary metabolites that stops or inhibits the growth of other micro-organisms *in-vitro* or *in-vivo* selectively. However, over the past few decades, the primary health care of humans are under threat due to emergence of drug-resistant bacteria, which makes it is essential to investigate newer drugs with lesser resistance. It has been known that drugs derived from natural sources play significant roles in the prevention and treatment of human diseases. Moreover, natural products of higher plants can be new source of antimicrobial agents with novel mechanisms of action. Therefore, the effects of plant extracts on bacteria have been studied by researchers worldwide and most of them on ethno medicinal plants in India.

Elytraria acaulis is a stem less perennial herb belonging to *acanthaceae* family of plant kingdom which is a small shrub that grows in shady dry places. Asian is a stem less perennial herb with one to several unbranched flowering stems; up to 30cm. Flowers are borne in spikes held in tight, overlapping bracts. This plant is often found on often on rocky or sandy soils. It is a traditional herb, is the whole plant is used for medicinal purposes^[1,2]. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine^[4]. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional

therapies of 80% of the world's population. The pharmacological industries have produced a number of new antibiotics; however, resistance to these drugs by microorganisms has increased due to their genetic ability to transmit and acquire resistance to synthetic drugs^[5]. The microorganisms which are used to check the anti-microbial activity are *Escherichia coli*, *Salmonella typhi*, and *klebsiella*^[6]. All these microbes are effective pathogens and cause numerous diseases in human beings. As mentioned earlier, due to prolong use of synthetic antibiotics, these microbes have gained resistance because of which infection/diseases caused by these have become difficult to cure.

II. MATERIALS AND METHODS

Chemicals: Nutrient Broth, Nutrient Agar, methanol, ethyl acetate, ethanol etc., were obtained from Hi Media, Mumbai, India. The standard reference antibiotic is Penicillin.

Microorganisms: The different strains of MRSA infected patients, like *Escherichia coli*, *Klebsiella Pneumoniae*, *Salmonellatyphi*, *Proteus Vulgaris*, and *Bacillus Subtilis* (ATCC 8739) at Global Hospital, Hyderabad. All the four cultures were maintained in Muller Hinton broth as well as on agar slants at 4°C.

2.1. Preparation of solvent extracts from different parts of plant material

Fresh leaves and stem of *Elytraria acaulis* were procured from local gardens at Hyderabad, India. The collected material was washed

thoroughly with distilled water and freeze dried. The dried samples were ground to powder and stored in tight container at -20°C until further use. Then the processed plant materials were subjected to fractionation using hexane, chloroform, ethanol, ethyl acetate, methanol and water in the order of increasing in polarity [7]. The extraction procedure was carried out at room temperature for 72h using mass to volume ratio of 1:10. Plant samples were then subjected to sequential extraction followed by Chloroform (C), Hexane (H) Ethyl acetate (EA), Methanol (M), and finally with Ethanol (E). By using a Rotary Evaporator each solvent present in the extracted material was evaporated at its respective boiling point. The solvent free extracts were dissolved in DMSO and were screened for their activity against the selected multi drug resistant pathogens [8].

2.2. Standardized bacterial colony numbers

In order to ensure that the same number of bacteria was always used, a set of bacterial growth curves was established in the laboratory using the method described by Cappuccino & Sherman (2005) for each bacterial strain prior to the evaluation of antimicrobial activities. From these curves, we determined the optical density (OD) at 600nm that corresponded to the desired number of colony forming units (CFU) [9].

2.3. Preparation of Test samples

One hundred milligrams (100mg) of plant extracts was dissolved in 1ml Dimethyl Sulfoxide (DMSO) while 1mg of penicillin was dissolved in 1ml of sterile deionised water. Sample prepared with ten microliters of 100mg/ml plant extract (equivalent to 1mg dose), 10µl of 1mg/ml penicillin (each equivalent to 10µg dose).

2.4. Antimicrobial testing Methods

2.4.1. Well-in Agar (WA)

Well-in agar assay was carried out according to the method with some modifications. Bacteria colonies from plates were grown in NB until they reached their specific OD at 600 nm to give starting inoculums of 1×10^8 bacteria/ml. Nutrient agar plates were each divided into quadrants and labeled accordingly. One hundred micro liters of inoculums, equivalent to 10^7 CFU, was mixed with 6ml of molten soft NA (to ensure even distribution of bacteria) and poured immediately onto the base layer of NA. The plates were left to solidify for 10min. A sterilized 5mm borer was used to make holes in the centre of the divided areas. The bottom of the well was then sealed with molten soft agar. Ten micro liters of each of the test samples [plant extracts and penicillin (It is used as positive control) & DMSO

(used as negative control)] was then pipette into the holes.

2.4.2. Bacterial growth condition

Acquisition Plates with bacteria and test samples were incubated at 37 °C for 16 to 18 hr after which the inhibition diameter (ID) was measured using a caliper. Each experiment was carried out on at least three separate occasions.

2.4.3. Minimum Inhibitory Concentration & Maximum Bactericidal Concentration

The minimum inhibitory concentration and maximum bactericidal concentration were determined by the broth dilution method with some modifications [10]. 4ml nutrient broth was dispensed in 6 test tubes. Into the first tube add 1ml of plant extract and then serially dilute. The stock concentration of plant extract was 5mg/ml and it was diluted up to 0.004 mg/ml. Then add 0.1ml of 24 hr old bacterial culture in all the tubes and incubate it for 24 hr at 37°C. After incubation MIC & MBC were calculated by comparing it with blanks (positive control). The lowest concentration that did not show any visible growth after macroscopic evaluation was considered as MIC. After the determination of MIC, the tubes which did not show any visible growth was diluted 100 fold with drug free NB and incubated at 37°C for 48 hr. The lowest concentration of the tube that did not show any visible growth was considered as the maximum bactericidal concentration (MBC) [11]. The assays were performed in triplicates.

III. Results And Discussion

The results of the antimicrobial activity of the *Elytraria* plant extracts are shown in Table 1. The results shows that the plant extracts were effective against both gram positive and gram negative organisms. The zones obtained from chloroform and methanol extracts were of almost equal diameter of all the test organisms except for *B.subtilis* which was found to be resistant. *B.subtilis* was only susceptible to ethyl acetate extract of the plant. The highest activity (diameter of zone of Inhibition 20 ± 0.31 mm) was demonstrated by the Hexane extract against *P.vul.*, while the lowest activity (11 ± 0.20 mm) was demonstrated by the Methanol extract against *K.pneu.*

Results of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are shown in Table 2. The minimum inhibitory concentration (MIC) of the Methanol and Hexane extracts for different organisms ranged between 0.078 and 0.312 mg/ml, while that of the remaining extracts MIC are 0.312 mg/ml. Also the MIC of Penicillin control ranged between 0.004 to

0.019 mg/ml, Methanol and Hexane extracts were more active than other extracts. The minimum bactericidal activity (MBC) of the extract for different bacteria ranged between 0.312 to 1.25 mg/ml, Methanol and Hexane extracts were more active than other extracts. This may be attributed to the presence of soluble phenolic and polyphenolic compounds^[16]. The inhibitory effect of the extract of *Elytraria Acaulis* against pathogenic bacterial strains can introduce the plant as a potential candidate for drug development for the treatment of ailments caused by these pathogens.

IV. CONCLUSION

In view of the continuous rise of antibiotic resistant bacterial strains, the present study was undertaken to analyze antibacterial activity of the aerial extracts of *Elytraria acaulis* and the findings clearly indicate that it has profound antimicrobial activity against the pathogenic strains. These results are supportive to the traditional use of *Elytraria acaulis* against multidrug resistant parasites of pathogenic strains. The demonstration of broad spectrum of antibacterial activity by *Elytraria acaulis* may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of gastrointestinal, urinary tract and wound infections and typhoid fever. The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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	EA	Hexane	Ethanol	Chloroform	Methanol	Pencillin
<i>E.coli</i>	13±0.63	20±0.31	12±0.20	14±0.30	---	21±0.11
<i>P.vul</i>	15±0.06	14±0.30	18±0.28	13±0.05	15±0.17	19±0.26
<i>B.sub</i>	16±0.06	---	---	---	---	22±0.15
<i>S.typhi</i>	16±0.20	14±0.06	14±0.25	13±0.11	15±0.03	21±0.11
<i>K.pneu</i>	15±0.03	16±0.03	13±0.24	13±0.17	11±0.20	21±0.26

Table1: Results of antimicrobial activities of plant extracts of *Elytraria acaulis* in different Solvents

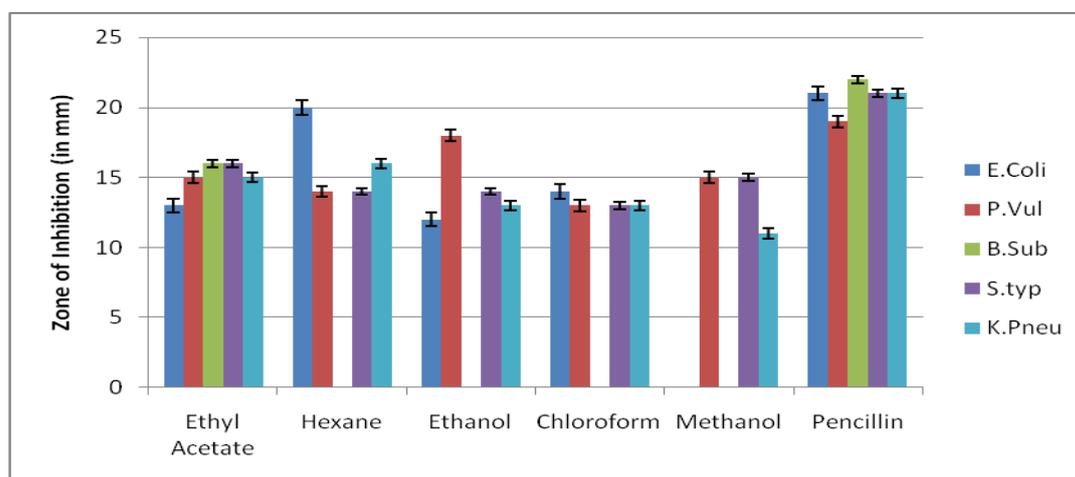


Fig1: Graphical representation of Antimicrobial activities of *Elytraria acaulis* Plant extracts in different solvents

Strains	Chloroform	Hexane	Methanol	Ethanol	Ethyl Acetate	Pencillin
	MIC	MIC	MIC	MIC	MIC	MIC
<i>E.coli</i>	0.312	0.312	-	0.312	0.312	0.019
<i>P.vul.</i>	0.312	0.078	0.312	0.312	0.312	0.019
<i>B.sub.</i>	-	-	-	-	0.312	0.019
<i>S.typhi</i>	0.312	0.312	0.078	0.312	0.312	0.004
<i>K.pneu.</i>	0.312	0.312	0.312	0.312	0.312	0.019

Table2:- Determination of Minimum inhibitory concentration of different extracts of *Elytraria acaulis*

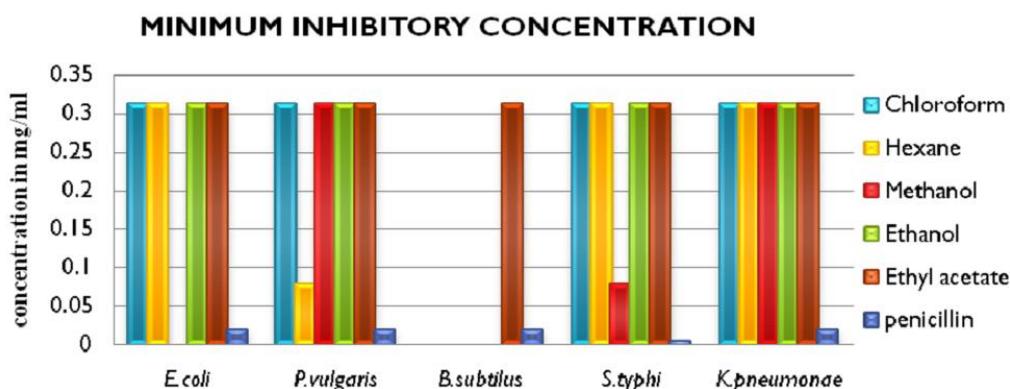


Fig2: Graphical representation of MIC values obtained for all the five extracts of *Elytraria acaulis* against susceptible test organisms.

Table3:-Determination of Maximum Bactericidal Concentration of different extracts of *Elytraria acaulis*

Strains	Chloroform	Hexane	Methanol	Ethanol	Ethyl Acetate	Penicillin
	MBC	MBC	MBC	MBC	MBC	MBC
<i>E.coli</i>	1.25	1.25	-	1.25	1.25	0.078
<i>P.vul</i>	1.25	0.312	1.25	1.25	1.25	0.078
<i>B.sub</i>	-	-	-	-	1.25	0.078
<i>S.typhi</i>	1.25	1.25	0.312	1.25	1.25	0.0195
<i>K.pneu</i>	1.25	1.25	1.25	1.25	1.25	0.078

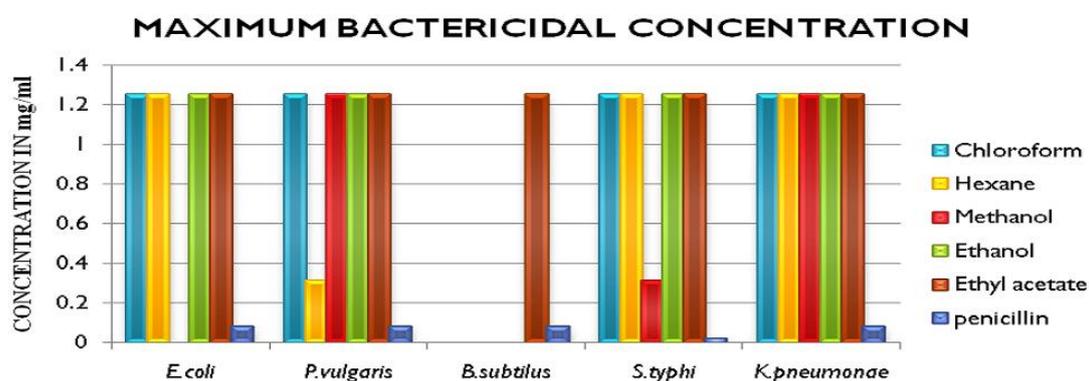


Fig 3:-Graphical representation of MBC values obtained for all the five extracts of *Elytraria acaulis* against susceptible test organisms.