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Screening of antimicrobial and antioxidative activities of the plant extracts of *Elytraria acaulis*

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ABSTRACT

Antimicrobial and Antioxidative activity of the aerial parts of the *Elytraria acaulis* a stem less perennial herb of *Acantheceae* 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. The free radical scavenging and antioxidant activity (AA) of the aerial extracts of *Elytraria acaulis* in different organic solvents was also assayed by DPPH assay, the aerial extracts of *Elytraria acaulis* has shown significant Anti oxidant activity. Hence further studies on this plant will enable elucidation of its therapeutic properties and medicinal applications.

Key words: Aerial parts, Antimicrobial activities, Antioxidant assays, *Elytraria acaulis,* Organic solvents, Pathogenic bacterial infections.

1. 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.

On the other hand, antioxidants are substances that protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and prevent the damage which may lead to cancer. Examples of antioxidants include beta-carotene, glutathione, lycopene, vitamins C, E, A well as enzymes such as catalase, superoxide dismutase and various peroxidases and other substances (Burits M et al.). Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly for treatments of stroke and neurodegenerative diseases (Das NP et al.). Antioxidants are also widely present as ingredients in dietary supplements maintaining health and preventing diseases such as cancer and coronary heart disease. Although, initial studies suggested that antioxidant supplements might promote health, later, large clinical trials did not detect any benefit and suggested instead that excess supplementation may be harmful. Free-radicals are very unstable and react quickly with other compound by abstracting its electron to attain stability. When the attacked molecule loses its electron, it becomes a free-radical itself, resulting in the disruption of the substance especially in fatty foods. Environmental factors such as pollution, radiation, cigarette smoking and herbicides can also spawn free-radicals in the body (Hamid AA et al.).

Elytraria acaulis is a stem less perennial herb belonging to *acantheceae* 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. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine (Kalemba D et al.). 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 (Karthikumar S et al.). The microorganisms which are used to check the anti-microbial activity are *Escherichia coli, Salmonella typhi, and klebsiella* (Marja P et al.). 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.

2. MATERIALS AND METHODS

2.1. General

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

Microorganisms: The different strains of MRSA infected patients like that etc., *Klebsiella, salmonella typhii,* and *Escherichia coli* (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.

2.2. 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 ethanol, ethyl acetate, methanol and water in the order of increasing in polarity (Mukhrizha etal.). 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 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 (Natarajan D et al.).

2.3. 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) (Nayan R et al.).

2.4. Preparation of test samples

One hundred milligrams (100mg) of plant extracts was dissolved in 1ml dimethyl sulfoxide (DMSO) while 1mg of streptomycin was dissolved in 1ml sterile deionised water. Ten microliters of 100mg/ml plant extract (equivalent to 1mg dose), 10µl of 1mg/ml streptomycin (each equivalent to 10µg dose).

2.5. Antimicrobial testing methods

2.5.1. 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 10^7CFU, 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, streptomycin or penicillin (these were used as positive controls) & DMSO (used as negative control)] was then pipette into the holes.

2.5.2. Bacterial growth condition

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

2.5.3. MIC and MBC

The minimum inhibitory concentration and maximum bactericidal concentration were determined by the broth dilution method with modification done (Peter J et al.). 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 hrs old bacterial culture in all the tubes and incubate it for 24 hrs 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 hrs. The lowest concentration of the tube that did not show any visible growth was considered as the maximum bactericidal concentration (MBC) (Roberto et al.). The assays were performed in triplicates.

2.6. Anti-oxidant activity

2.6.1. DPPH Assay

The free radical-scavenging activity of the extract of *Elytraria acaulis* all three extracts was determined as previously described by Burits and Bucar with some modification. The capacity of extracts to scavenge lipid-soluble 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, which results in bleaching of yellow color exhibited by stable DPPH radical, is monitored at an absorbance of 517nm. Briefly, 1.0 ml of extracts (0.05 – 1.0 mg/ml) and ascorbic acid (20–100µg/ml) in ethanol were added to 4 ml of 0.004% methanolic solution of DPPH. After incubation for 20 min at room temperature in the dark, absorbance was read against a blank at 517 nm. Tests were carried out in triplicate. The ability of extracts and ascorbic acid to scavenge DPPH radical was calculated using the following equation (Sathish Babu P et al.):

Radical scavenging activity (%) = $[A_0 – A_1/ A_0] \times 100$.

Where A_0 was absorbance of negative control (containing all reagents except test compounds) at 517 nm and A_1 was absorbance of the extracts or ascorbic acid at 517 nm. DPPH scavenging activity of extracts and standard was expressed as IC_{50}, which was interpolated from a graph constructed, using percent inhibition (Y-axis) against concentration (X-axis) of extracts and standards.

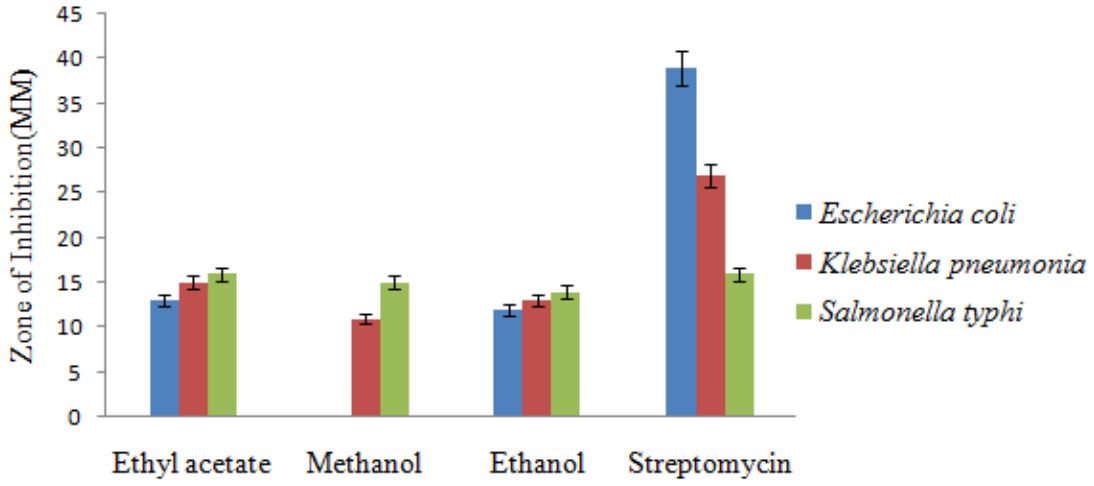

Figure 1

Antibacterial activity of agar well effect of *Elytraria acaulis* extract with different solvents in comparison with streptomycin (a positive control antibiotic).

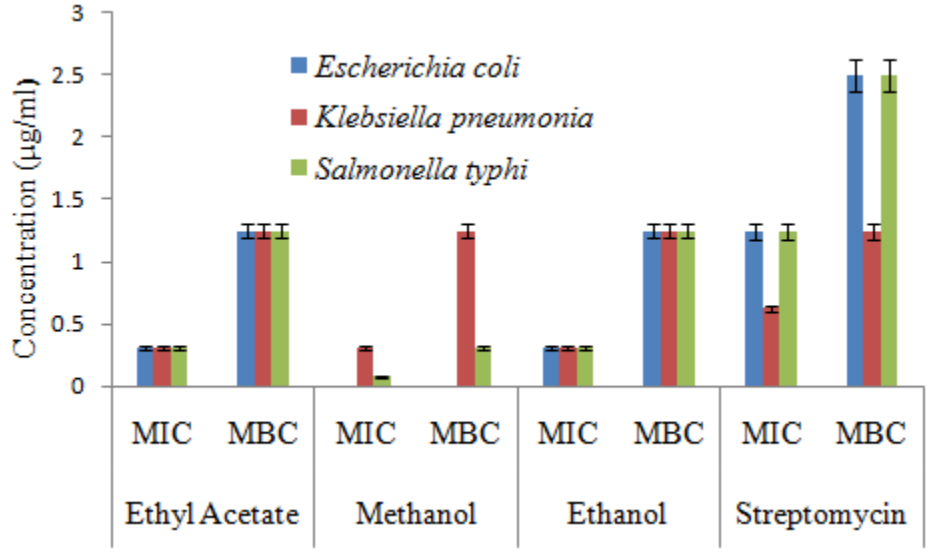

Figure 2

Antibacterial activity of MIC & MBC effect of different solvents and *Elytraria acaulis* extract of arial parts in comparison with streptomycin (a positive control antibiotic)

3. RESULTS AND DISCUSSION

In Antimicrobial activity the zones obtained from chloroform and methanol extracts were of almost equal diameter of all the test organisms except for *B.substilus* which was found to be resistant. *B.subtilus* was only susceptible to ethyl acetate extract of the plant. To check the maximum bactericidal concentration, one fold higher concentration of MIC was considered as MBC. The MBC values were in the range of 0.312mg/ml-1.25mg/ml. Since the chemical composition and structures of active extract components are important factors governing the efficacy of natural antioxidants, the antioxidant activity of an extract could not be explained on the basis of their phenolic content, which also needs their characterization. These results from various free radical-scavenging systems

revealed that the *Elytraria acualis* had significant antioxidant activity and free radical-scavenging activity (Sreejayan N et al.). The compounds such as flavonoids and polyphenols, which contain hydroxyls, are responsible for the radical scavenging effect of plants (Das NP et al, 1990) (Susana Johann etal.). Among the three extracts and standard tested for the in vitro antioxidant activity using the DPPH method and the crude methanolic, ethanolic and ethyl acetate extracts of *Elytraria acualis* showed antioxidant activity, with maximum of 57% (at 60µg/ml); 57.47% (40µg/ml) and 50.93% (60µg/ml) respectively when compared to that of the standard (ascorbic acid) that gave 45.79% (at 100µg/ml) activity. The results indicate that the antioxidant activity of the crude extract of *Elytraria acualis* is higher than that of ascorbic acid. Thus methanolic extract of leaves was a good antioxidant than other extracts of ethyl acetate & chloroform. Furthermore the antioxidant activity was associated with this result is due to high level of polyphenol and vitamins A and C. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability.

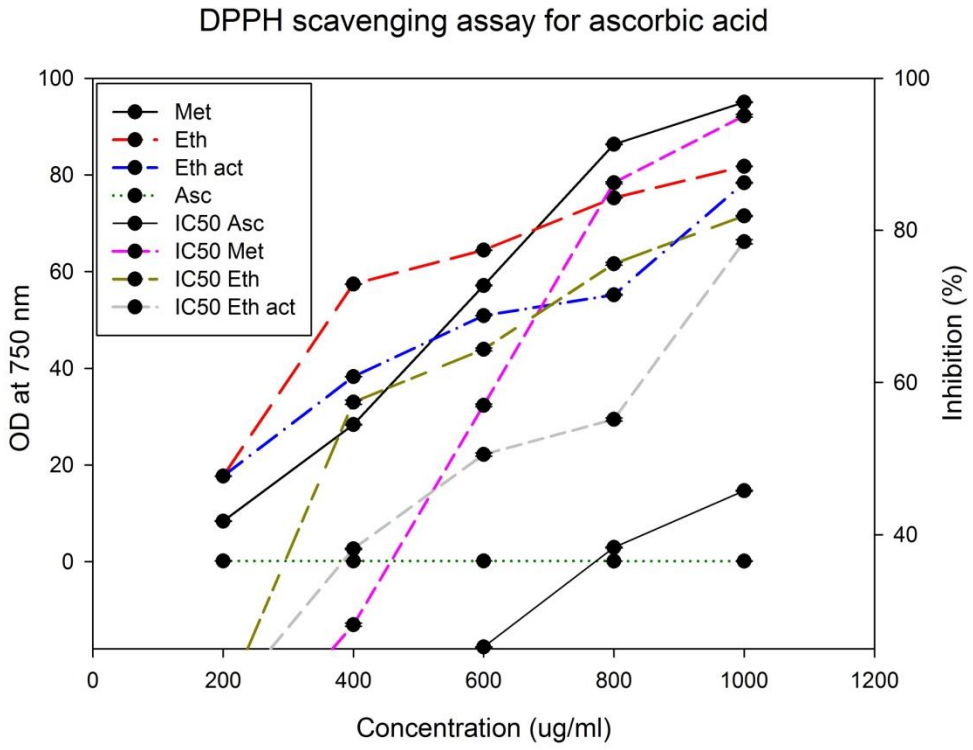

Figure 3

The free radical scavenging activity of different solvents of the plant extracts of *Elytraria acaulis* by DPPH assay method

Table 1

The Antibacterial activity of agar well effect of Elytraria *acaulis* plant aerial extract against pathogenic bacterial strains

Name of the organisms	Diameter of zone inhibition (mm) (10mg/ml Concentration)			
	Ethyl acetate	Methanol	Ethanol	Streptomycin
Escherichia. coli	13 ±05	No Inhibition activity	12 ± 02	39± 02
Klebsiella pneumonia	15 ± 03	11 ± 05	13 ± 05	27± 02
Salmonella typhi	16 ±02	15 ±03	14 ±04	16± 01

Table 2

Determination of Minimum inhibitory and bactericidal concentrations of *Elytraria acaulis* plant aerial extract and Streptomycin effect on pathogenic bacterial strains

Plant extract 10mg/ml		*Escherichia. coli*	*Klebsiella pneumonia*	*Salmonella Typhi*
Ethyl Acetate	MIC	0.312±003	0.312± 005	0.312± 002
	MBC	1.25± 004	1.25± 004	1.25± 002
Methanol	MIC	-	0.312± 002	0.078± 003
	MBC	-	1.25± 003	0.312± 005
Ethanol	MIC	0.312±003	0.312± 004	0.312± 005
	MBC	1.25± 001	1.25± 002	1.25± 003
Streptomycin	MIC	0.04± 003	0.008± 004	0.004± 005
	MBC	0.08± 003	0.032± 004	0.032± 005

Table 3

The free radical scavenging activity effect of different solvents of the plant extracts of *Elytraria acaulis* by DPPH assay method

Concentration (µg/ml)	Methanol	Ethanol	Ethyl acetate	Ascorbic acid	Inhibition (%)
20	8.41±0.92	17.75±1.34	17.75±1.32	0.192±1.23	10.28±2
40	28.50±1.34	57.47±1.65	38.31±1.45	0.173±1.34	19.15±2
60	57.00±0.87	64.48±1.54	50.93±1.34	0.159±1.54	25.17±2
80	86.44±1.76	75.23±0.54	55.00±0.67	0.132±1.31	38.31±2
100	95.00±0.95	81.77±0.45	78.50±1.65	0.116±1.55	45.79±2

4. CONCLUSION

In view of the continuous rise of antibiotic resistant bacterial strains, the present study was undertaken to analyze antibacterial activity and Anti oxidants of the aerial extracts of *Elytraria acaulis* and the findings clearly indicate that *Elytraria acaulis* 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. Many substances consumed by man either through foods, drinks and inhalation, even effect of exogenous material (ultraviolet radiation) on the skin may be destructive to the health and thus, shortening the life span of man. Free radicals when generated in the body system of man causes damage to which eventually leads to death at shorter time. Continuous usage of the same vegetable oil which is not even properly stored and re-using the already fried oil (rancid) lead to generation of free radicals through lipid peroxidation. Further research is being carried out in our laboratory for the determination of mechanism of action, toxicological studies and formulation of the compound.

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